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(57) Abstract

A biosynthetic method for producing vitamin C (ascorbic acid, L-ascorbic acid, or AA) is disclosed. Such a method includes fermentation of a genetically modified microorganism or plant to produce L-ascorbic acid. In particular, the present invention relates to the use of microorganisms and plants having at least one genetic modification to increase the action of an enzyme involved in the ascorbic acid biosynthetic pathway. Included is the use of nucleotide sequences encoding epimerases, including the endogenous GDP-D-mannose:GDP-L-galactose epimerase from the L-ascorbic acid pathway and homologues thereof for the purposes of improving the biosynthetic production of ascorbic acid. The present invention also relates to genetically modified microorganisms, such as strains of microalgae, bacteria and yeast useful for producing L-ascorbic acid, and to genetically modified plants, useful for producing consumable plant food products.

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VITAMIN C PRODUCTION IN MICROORGANISMS AND PLANTS

FIELD OF THE INVENTION

The present invention relates to vitamin C (L-ascorbic acid) production using genetically modified microorganisms and plants. In particular, the present invention relates to the use of nucleotide sugar epimerase enzymes for the biological production of ascorbic acid in plants and microorganisms.

BACKGROUND OF THE INVENTION

Nearly all forms of life, both plant and animal, either synthesize ascorbic acid (vitamin C) or require it as a nutrient. Ascorbic acid was first identified to be useful as a dietary supplement for humans and animals for the prevention of scurvy. Ascorbic acid, however, also affects human physiological functions such as the adsorption of iron, cold tolerance, the maintenance of the adrenal cortex, wound healing, the synthesis of polysaccharides and collagen, the formation of cartilage, dentine, bone and teeth, the maintenance of capillaries, and is useful as an antioxidant.

For use as a dietary supplement, ascorbic acid can be isolated from natural sources, such as rosehips, synthesized chemically through the oxidation of L-sorbose, or produced by the oxidative fermentation of calcium D-gluconate by *Acetobacter suboxidans*. Considine, "Ascorbic Acid," *Van Nostrand's Scientific Encyclopedia*, Vol. 1, pp. 237-238, (1989). Ascorbic acid (predominantly intracellular) has also been obtained through the fermentation of strains of the microalga, *Chlorella pyrenoidosa*. See U.S. Patent No. 5,001,059 by Skatrud, which is assigned to the assignee of the present application. It is believed that ascorbic acid is produced inside the chloroplasts of photosynthetic microorganisms and functions to neutralize energetic electrons produced during photosynthesis. Accordingly, ascorbic acid production is known in photosynthetic organisms as a protective mechanism.

Therefore, products and processes which improve the ability to biosynthetically produce ascorbic acid are desirable and beneficial for the improvement of human health.

SUMMARY OF THE INVENTION

One embodiment of the present invention relates to a method for producing ascorbic acid or esters thereof in a microorganism. The method includes the steps of: (a)

culturing a microorganism having a genetic modification to increase the action of an enzyme selected from the group of hexokinase, glucose phosphate isomerase, phosphomannose isomerase, phosphomannomutase, GDP-D-mannose pyrophosphorylase, GDP-D-mannose:GDP-L-galactose epimerase, GDP-L-galactose phosphorylase, L-galactose-1-P-phosphatase, L-galactose dehydrogenase, and/or L-galactono-γ-lactone dehydrogenase; and (b) recovering the ascorbic acid or esters produced by the microorganism. Preferably, the genetic modification is a genetic modification to increase the action of an enzyme selected from the group of GDP-D-mannose:GDP-L-galactose epimerase, GDP-L-galactose phosphorylase, L-galactose-1-P-phosphatase, L-galactose dehydrogenase, and/or L-galactono-γ-lactone dehydrogenase. In one embodiment of the method of the present invention, the microorganism further includes a genetic modification to decrease the action of an enzyme having GDP-D-mannose as a substrate, other than GDP-D-mannose:GDP-L-galactose epimerase. Such a genetic modification can include, for example, a genetic modification to decrease the action of GDP-D-mannose-dehydrogenase.

In one embodiment, the genetic modification is a genetic modification to increase the action of an epimerase that catalyzes conversion of GDP-D-mannose to GDP-L-galactose, which can include GDP-D-mannose:GDP-L-galactose epimerase. In one embodiment, the epimerase binds NADPH. In one embodiment of this method, the genetic modification includes transformation of the microorganism with a recombinant nucleic acid molecule that expresses the epimerase. Such an epimerase can have a tertiary structure that substantially conforms to the tertiary structure of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws. Preferably, the epimerase has a structure having an average root mean square deviation of less than about 2.5 Å, and more preferably less than about 1 Å, over at least about 25% of Cα positions of the tertiary structure of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws.

In one embodiment, the epimerase comprises a substrate binding site having a tertiary structure that substantially conforms to the tertiary structure of the substrate binding site of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by

atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws. Such a substrate binding site preferably has a tertiary structure with an average root mean square deviation of less than about 2.5 Å over at least about 25% of Cα positions of the tertiary structure of a substrate binding site of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws.

In another embodiment, the epimerase comprises a catalytic site having a tertiary structure that substantially conforms to the tertiary structure of the catalytic site of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws. Such a catalytic site preferably has a tertiary structure with an average root mean square deviation of less than about 1 Å over at least about 25% of Cα positions of the tertiary structure of a catalytic site of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws. The catalytic site preferably includes the amino acid residues serine, tyrosine and lysine and in one embodiment, the tertiary structure positions of the amino acid residues serine, tyrosine and lysine substantially conform to tertiary structure positions of residues Ser107, Tyr136 and Lys140, respectively, as represented by atomic coordinates in Brookhaven Protein Data Bank Accession Code 1bws.

In yet another embodiment of this method, the epimerase comprises an amino acid sequence that aligns with SEQ ID NO:11 using a CLUSTAL alignment program, wherein amino acid residues in the amino acid sequence align with 100% identity with at least about 50%, and in another embodiment with at least about 75%, and in yet another embodiment with at least about 90% of non-Xaa residues in SEQ ID NO:11. In another embodiment, the epimerase comprises an amino acid sequence having at least 4 contiguous amino acid residues that are 100% identical to at least 4 contiguous amino acid residues of an amino acid sequence selected from the group of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8 and SEQ ID NO:10. In yet another embodiment, the recombinant nucleic acid molecule comprises a nucleic acid sequence comprising at least about 12 contiguous nucleotides having 100% identity with at least about 12

contiguous nucleotides of a nucleic acid sequence selected from the group of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7 and SEQ ID NO:9.

In yet another embodiment of this method of the present invention, the epimerase comprises an amino acid sequence having a motif: Gly-Xaa-Xaa-Gly-Xaa-Xaa-Gly. In yet another embodiment, the recombinant nucleic acid molecule comprises a nucleic acid sequence that is at least about 15% identical, and in another embodiment, at least about 20% identical, and in another embodiment, at least about 25% identical, to a nucleic acid sequence selected from the group of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7 and SEQ ID NO:9, as determined using a Lipman-Pearson method with Lipman-Pearson standard default parameters.

In yet another embodiment of this method of the present invention, the recombinant nucleic acid molecule comprises a nucleic acid sequence that hybridizes under stringent hybridization conditions to a nucleic acid sequence encoding a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase. The nucleic acid sequence encoding the GDP-4-keto-6-deoxy-D-mannose epimerase/reductase includes nucleic acid sequences selected from the group of SEQ ID NO:1, SEQ ID NO:3 and SEQ ID NO:5, and the GDP-4-keto-6-deoxy-D-mannose epimerase/reductase can include an amino acid sequence selected from the group of SEQ ID NO:2, SEQ ID NO:4 and SEQ ID NO:6.

In one embodiment of the method of the present invention, the microorganism is selected from the group of bacteria, fungi and microalgae. In one embodiment, the microorganism is acid-tolerant. Preferred bacteria include, but are not limited to *Azotobacter* and *Pseudomonas*. Preferred fungi include, but are not limited to, yeast, including, but not limited to *Saccharomyces* yeast. Preferred microalgae include, but are not limited to, microalgae of the genera *Prototheca* and *Chlorella*, with microalgae of the genus *Prototheca* being particularly preferred.

In yet another embodiment of the method of the present invention, the microorganism is acid-tolerant and the step of culturing is conducted at a pH of less than about 6.0, and more preferably, at a pH of less than about 5.5, and even more preferably, at a pH of less than about 5.0. The step of culturing can be conducted in a fermentation medium that comprises a carbon source other than D-mannose in one embodiment, and

in another embodiment, the step of culturing is conducted in a fermentation medium that comprises glucose as a carbon source.

In yet another embodiment of the present method, the step of culturing is conducted in a fermentation medium that is magnesium (Mg) limited. Preferably, the step of culturing is conducted in a fermentation medium that is Mg limited during a cell growth phase. In one embodiment, the fermentation medium includes less than about 0.5 g/L of Mg during a cell growth phase, and more preferably, less than about 0.2 g/L of Mg during a cell growth phase, and even more preferably, less than about 0.1 g/L of Mg during a cell growth phase.

Another embodiment of the present invention relates to a microorganism for producing ascorbic acid or esters thereof. The microorganism has a genetic modification to increase the action of an enzyme selected from the group of hexokinase, glucose phosphate isomerase, phosphomannose isomerase, phosphomannomutase, GDP-D-mannose pyrophosphorylase, GDP-D-mannose:GDP-L-galactose epimerase, GDP-L-galactose phosphorylase, L-galactose-1-P-phosphatase, L-galactose dehydrogenase, and/or L-galactono-γ-lactone dehydrogenase. Preferably, the genetic modification is a genetic modification to increase the action of an enzyme selected from the group of GDP-D-mannose:GDP-L-galactose epimerase, GDP-L-galactose phosphorylase, L-galactose-1-P-phosphatase, L-galactose dehydrogenase, and/or L-galactono-γ-lactone dehydrogenase, and even more preferably, to increase the action of GDP-D-mannose:GDP-L-galactose epimerase.

In one embodiment, the microorganism has been genetically modified to express a recombinant nucleic acid molecule encoding an epimerase that catalyzes conversion of GDP-D-mannose to GDP-L-galactose, wherein the epimerase has a tertiary structure having an average root mean square deviation of less than about 2.5 Å over at least about 25% of Cα positions of the tertiary structure of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws. In another embodiment, the microorganism has been genetically modified to express a recombinant nucleic acid molecule encoding an epimerase that catalyzes conversion of GDP-D-mannose to GDP-L-galactose, wherein the

CLUSTAL alignment program, wherein amino acid residues in the amino acid sequence align with 100% identity with at least about 50% of non-Xaa residues in SEQ ID NO:11. Preferred microorganisms are disclosed as for the method discussed above.

Yet another embodiment of the present invention relates to a plant for producing ascorbic acid or esters thereof. Such a plant has a genetic modification to increase the action of an enzyme selected from the group of hexokinase, glucose phosphate isomerase, phosphomannose isomerase, phosphomannomutase, GDP-D-mannose pyrophosphorylase, GDP-D-mannose:GDP-L-galactose epimerase, GDP-L-galactose phosphorylase, L-galactose-1-P-phosphatase, L-galactose dehydrogenase, and/or L-galactono-γ-lactone dehydrogenase. In a preferred embodiment, the genetic modification is a genetic modification to increase the action of an enzyme selected from the group of GDP-D-mannose:GDP-L-galactose epimerase, GDP-L-galactose phosphorylase, L-galactose-1-P-phosphatase, L-galactose dehydrogenase, and/or L-galactono-γ-lactone dehydrogenase, and in a more preferred embodiment, the genetic modification is a genetic modification to increase the action of GDP-D-mannose:GDP-L-galactose epimerase.

In one embodiment, the plant further comprises a genetic modification to decrease the action of an enzyme having GDP-D-mannose as a substrate other than GDP-D-mannose:GDP-L-galactose epimerase. Such a genetic modification includes a genetic modification to decrease the action of GDP-D-mannose-dehydrogenase. Such a plant also includes a plant that has been genetically modified to express a recombinant nucleic acid molecule encoding an epimerase that catalyzes conversion of GDP-D-mannose to GDP-L-galactose, wherein the epimerase has a tertiary structure having an average root mean square deviation of less than about 2.5 Å over at least about 25% of Cα positions of the tertiary structure of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws. In another embodiment, such a plant has been genetically modified to express a recombinant nucleic acid molecule encoding an epimerase that catalyzes conversion of GDP-D-mannose to GDP-L-galactose, wherein the epimerase comprises an amino acid sequence that aligns with SEQ ID NO:11 using a CLUSTAL alignment program, wherein amino acid residues in the amino acid sequence align with 100% identity with at least about 50% of non-Xaa residues in SEQ ID NO:11.

In one embodiment, a plant for producing ascorbic acid or esters thereof according to the present invention is a microalga. Preferred microalgae include, but are not limited to microalgae of the genera *Prototheca* and *Chlorella*, with microalga of the genus *Prototheca* being particularly preferred. In another embodiment, the plant is a higher plant, with consumable higher plants being more preferred.

BRIEF DESCRIPTION OF THE FIGURES

Fig. 1A is a schematic drawing of the pathway from glucose to GDP-D-mannose in plants.

Fig. 1B is a schematic drawing of the pathway from GDP-D-mannose to L-galactose-1-phosphate in plants.

Fig. 1C is a schematic drawing of the pathway from L-galactose to L-ascorbic acid in plants.

Fig. 2A is a schematic drawing of selected carbon flow from glucose in *Prototheca*.

Fig. 2B is a schematic drawing of selected carbon flow from glucose in *Prototheca*.

Fig. 3 is a schematic drawing that shows the lineage of mutants derived from *Prototheca moriformis* ATCC 75669, and their ability to produce L-ascorbic acid.

Fig. 4 is a bar graph illustrating the conversion of substrates by resting cells of strain NA45-3 following growth in media containing various magnesium concentrations and resuspension in media containing various magnesium concentrations.

Fig. 5 is a line graph showing the relationship between specific ascorbic acid formation in cultures of *Prototheca* strains and the specific activity of GDP-D-mannose:GDP-L-galactose epimerase in extracts prepared from cells harvested from the same cultures.

Fig. 6 is a line graph showing the relationship between specific epimerase activity and the degree of magnesium limitation in two strains, ATCC 75669 and EMS13-4.

Fig. 7 depicts the overall catalytic mechanism of GDP-D-mannose:GDP-L-galactose epimerase proposed by Barber (1979, *J. Biol. Chem.* 254:7600-7603).

Fig. 8A depicts the catalytic mechanism of GDP-D-mannose-4,6-dehydratase (converts GDP-D-mannose to GDP-4-keto-6-deoxy-D-mannose).

Fig. 8B depicts the catalytic mechanism of GDP-4-keto-6-deoxy-D-mannose epimerase/reductase (converts GDP-4-keto-6-deoxy-D-mannose to GDP-L-fucose) (Chang, et al., 1988, *J. Biol. Chem.* 263:1693-1697; Barber, 1980, *Plant Physiol.* 66:326-329).

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a biosynthetic method and production microorganisms and plants for producing vitamin C (ascorbic acid, L-ascorbic acid, or AA). Such a method includes fermentation of a genetically modified microorganism to produce L-ascorbic acid. In particular, the present invention relates to the use of nucleotide sequences encoding epimerases, including the endogenous GDP-D-mannose:GDP-L-galactose epimerase from the L-ascorbic acid pathway, as well as epimerases having structural homology (e.g., by nucleotide/amino acid sequence and/or tertiary structure of the encoded protein) to GDP-4-keto-6-deoxy-D-mannose epimerase/ reductases, or UDP-galactose 4-epimerases, for the purposes of improving the biosynthetic production of ascorbic acid. The present invention also relates to genetically modified microorganisms, such as strains of microalgae, bacteria and yeast useful for producing L-ascorbic acid, and to genetically modified plants, useful for producing consumable plant food products.

One embodiment of the present invention relates to a method to produce L-ascorbic acid by fermentation of a genetically modified microorganism. This method includes the steps of (a) culturing in a fermentation medium a microorganism having a genetic modification to increase the action of an enzyme selected from the group of hexokinase, glucose phosphate isomerase, phosphomannose isomerase, phosphomannomutase, GDP-mannose pyrophosphorylase, GDP-D-mannose:GDP-L-galactose epimerase, GDP-L-galactose phosphorylase, L-galactose-1-P-phosphatase, L-galactose dehydrogenase, and L-galactono-γ-lactone dehydrogenase; and (b) recovering L-ascorbic acid or esters thereof. The various enzymes in this list represent the enzymes involved in the vitamin C biosynthetic pathway in plants. It is uncertain at this time

whether the enzyme represented by GDP-L-galactose phosphorylase is actually a phosphorylase or a pyrophosphorylase (i.e., GDP-L-galactose pyrophosphorylase). Therefore, use of the term "GDP-L-galactose phosphorylase" herein refers to either GDP-L-galactose phosphorylase or GDP-L-galactose pyrophosphorylase. In one aspect of the invention, this method includes the step of culturing in a fermentation medium a microorganism having a genetic modification to increase the action of an epimerase that catalyzes conversion of GDP-D-mannose to GDP-L-galactose. This aspect of the present invention is discussed in detail below.

Another embodiment of the present invention relates to a genetically modified microorganism for producing L-ascorbic acid or esters thereof. Another embodiment of the present invention relates to a genetically modified plant for producing L-ascorbic acid or esters thereof. Both genetically modified microorganisms (e.g., bacteria, yeast, microalgae) and plants (e.g., higher plants, microalgae) have a genetic modification to increase the action of an enzyme selected from the group of hexokinase, glucose phosphate isomerase, phosphomannose isomerase, phosphomannomutase, GDP-mannose pyrophosphorylase, GDP-D-mannose:GDP-L-galactose epimerase, GDP-L-galactose phosphorylase, L-galactose-1-P-phosphatase, L-galactose dehydrogenase, and/or L-galactono-γ-lactone dehydrogenase. In a preferred embodiment, both genetically modified microorganisms (e.g., bacteria, yeast, microalgae) and plants (e.g., higher plants, microalgae) have a genetic modification to increase the action of an epimerase that catalyzes conversion of GDP-D-mannose to GDP-L-galactose. In one embodiment, the genetic modification includes the transformation of the microorganism or plant with the epimerase as described above.

To produce significantly high yields of L-ascorbic acid by the method of the present invention, a plant and/or microorganism is genetically modified to enhance production of L-ascorbic acid. As used herein, a genetically modified plant (such as a higher plant or microalgae) or microorganism, such as a microalga (*Prototheca, Chlorella*), *Escherichia coli*, or a yeast, is modified (i.e., mutated or changed) within its genome and/or by recombinant technology (i.e., genetic engineering) from its normal (i.e., wild-type or naturally occurring) form. In a preferred embodiment, a genetically modified plant or microorganism according to the present invention has been modified by

recombinant technology. Genetic modification of a plant or microorganism can be accomplished using classical strain development and/or molecular genetic techniques, include genetic engineering techniques. Such techniques are generally disclosed herein and are additionally disclosed, for example, in Sambrook et al., 1989, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Labs Press; Roessler, 1995, *Plant Lipid Metabolism*, pp. 46-48; and Roessler et al., 1994, in Bioconversion for Fuels, Himmel et al. eds., American Chemical Society, Washington D.C., pp 255-70). These references are incorporated by reference herein in their entirety.

In some embodiments, a genetically modified plant or microorganism can include a natural genetic variant as well as a plant or microorganism in which nucleic acid molecules have been inserted, deleted or modified, including by mutation of endogenous genes (e.g., by insertion, deletion, substitution, and/or inversion of nucleotides), in such a manner that the modifications provide the desired effect within the plant or microorganism. As discussed above, a genetically modified plant or microorganism includes a plant or microorganism that has been modified using recombinant technology.

As used herein, genetic modifications which result in a decrease in gene expression, an increase in inhibition of gene expression or inhibition of a gene product (i.e., the protein encoded by the gene), a decrease in the function of the gene, or a decrease in the function of the gene product can be referred to as inactivation (complete or partial), deletion, interruption, blockage, down-regulation, or decreased action of a gene. For example, a genetic modification in a gene which results in a decrease in the function of the protein encoded by such gene can be the result of a complete deletion of the gene encoding the protein (i.e., the gene does not exist, and therefore the protein does not exist), a mutation in the gene encoding the protein which results in incomplete or no translation of the protein (e.g., the protein is not expressed), or a mutation in the gene which decreases or abolishes the natural function of the protein (e.g., a protein is expressed which has decreased or no enzymatic activity).

Genetic modifications which result in an increase in gene expression or function can be referred to as amplification, overproduction, overexpression, activation, enhancement, addition, up-regulation or increased action of a gene. Additionally, a genetic modification to a gene which modifies the expression, function, or activity of the gene can

have an impact on the action of other genes and their expression products within a given metabolic pathway (e.g., by inhibition or competition). In this embodiment, the action (e.g., activity) of a particular gene and/or its product can be affected (i.e., upregulated or downregulated) by a genetic modification to another gene within the same metabolic pathway, or to a gene within a different metabolic pathway which impacts the pathway of interest by competition, inhibition, substrate formation, etc.

In general, a plant or microorganism having a genetic modification that affects L-ascorbic acid production has at least one genetic modification, as discussed above, which results in a change in the L-ascorbic acid production pathway as compared to a wild-type plant or microorganism grown or cultured under the same conditions. Such a modification in an L-ascorbic acid production pathway changes the ability of the plant or microorganism to produce L-ascorbic acid. According to the present invention, a genetically modified plant or microorganism preferably has an enhanced ability to produce L-ascorbic acid compared to a wild-type plant or microorganism cultured under the same conditions.

The present invention is based on the present inventors' discovery of the biosynthetic pathway for L-ascorbic acid (vitamin C) in plants and microorganisms. Prior to the present invention, the metabolic pathway by which plants produce L-ascorbic acid, was not completely elucidated. The present inventors have demonstrated that L-ascorbic acid production in plants, including L-ascorbic acid-producing microorganisms (e.g., microalgae), is a pathway which uses GDP-D-mannose and involves sugar phosphates and NDP-sugars. In addition, the present inventors have made the surprising discovery that both L-galactose and L-galactono-γ-lactone can be rapidly converted into L-ascorbic acid in L-ascorbic acid-producing microalgae, including *Prototheca* and *Chlorella pyrenoidosa*. The entire pathway for L-ascorbic acid production in plants is set forth in Figs. 1A-1C. More particularly, Fig. 1A shows that the production of L-ascorbic acid in plants proceeds through the production of mannose intermediates to GDP-D-mannose, followed by the conversion of GDP-D-mannose to GDP-L-galactose by GDP-D-mannose:GDP-L-galactose epimerase (also known as GDP-D-mannose-3,5-epimerase) (Fig. 1B), and then by the subsequent progression to L-galactose-1-P, L-galactose, L-galactonic acid (optional), L-galacto

also illustrates alternate pathways for the use of various intermediates, such as GDP-D-mannose. Certain aspects of this pathway have been independently described in a publication (Wheeler, et al., 1998, *Nature* 393:365-369), incorporated herein by reference in its entirety.

Points within the L-ascorbic acid production pathway which can be targeted by genetic modification to affect the production of L-ascorbic acid can generally be catagorized into at least one of the following pathways: (a) pathways affecting the production of GDP-D-mannose (e.g., pathways for converting a carbon source into GDP-D-mannose); (b) pathways for converting GDP-D-mannose into other compounds, (c) pathways associated with or downstream of the action of GDP-D-mannose:GDP-L-galactose epimerase, (d) pathways which compete for substrates involved in the production of any of the intermediates within the L-ascorbic acid production pathway, and in particular, with GDP-D-mannose, GDP-L-galactose, L-galactose-1-phosphate, L-galactose, L-galactono-γ-lactone, and/or L-ascorbic acid; and (e) pathways which inhibit production of any of the intermediates within the L-ascorbic acid production pathway, and in particular, with GDP-D-mannose, GDP-L-galactose, L-galactose-1-phosphate, L-galactose, L-galactono-γ-lactone, and/or L-ascorbic acid.

A genetically modified plant or microorganism useful in a method of the present invention typically has at least one genetic modification in the L-ascorbic acid production pathway which results in an enhanced production of L-ascorbic acid. In one embodiment, a genetically modified plant or microorganism has at least one genetic modification that results in: (a) an enhanced production of GDP-D-mannose; (b) an inhibition of pathways which convert GDP-D-mannose into compounds other than GDP-L-galactose; (c) an enhancement of action of the GDP-D-mannose:GDP-L-galactose epimerase; (d) an enhancement of the action of enzymes downstream of the GDP-D-mannose:GDP-L-galactose epimerase; (e) an inhibition of pathways which compete for substrates involved in the production of any of the intermediates within the L-ascorbic acid production pathway, and in particular, with GDP-D-mannose, GDP-L-galactose, L-galactose-1-phosphate, L-galactose, L-galactono-γ-lactone, and/or L-ascorbic acid; and (e) an inhibition of pathways which inhibit production of any of the intermediates within the L-ascorbic acid production pathway, and in particular, with GDP-D-mannose, GDP-L-

galactose, L-galactose-1-phosphate, L-galactose, L-galactono-γ-lactone, and/or L-ascorbic acid.

An enhanced production of GDP-D-mannose by genetic modification of the plant or microorganism can be achieved by, for example, overexpression of enzymes such as hexokinase, glucose phosphate isomerase, phosphomannose isomerase (PMI), phosphomannomutase (PMM) and/or GDP-D-mannose pyrophosphorylase (GMP). Inhibition of pathways which convert GDP-D-mannose to compounds other than GDP-L-galactose can be achieved, for example, by modifications which inhibit polysaccharide synthesis, GDP-D-rhamnose synthesis, GDP-L-fucose synthesis and/or GDP-D-mannuronic acid synthesis. An increase in the action of the GDP-D-mannose:GDP-L-galactose epimerase and of enzymes downstream of the epimerase in the L-ascorbic acid production pathway can be achieved by genetic modifications which include, but are not limited to: overexpression of the epimerase gene (i.e, by overexpression of a recombinant nucleic acid molecule encoding the epimerase gene or a homologue thereof (discussed in detail below), and/or by mutation of the endogenous or recombinant gene to enhance expression of the gene) and/or overexpression of genes downstream of the epimerase which encode subsequent enzymes in the L-ascorbic acid pathway. Finally, metabolic pathways which compete with or inhibit the L-ascorbic acid production pathway can be inhibited by deleting or mutating enzymes, substrates or products which either inhibit or compete for an enzyme, substrate or product in the L-ascorbic acid pathway.

As discussed above, a genetically modified plant or microorganism useful in the method of the present invention can have at least one genetic modification (e.g., mutation in the endogenous gene or addition of a recombinant gene) in a gene encoding an enzyme involved in the L-ascorbic acid production pathway. Such genetic modifications preferably increase (i.e., enhance) the action of such enzymes such that L-ascorbic acid is preferentially produced as compared to other possible end products in related metabolic pathways. Such genetic modifications include, but are not limited to, overexpression of the gene encoding such enzyme, and deletion, mutation, or downregulation of genes encoding competitors or inhibitors of such enzyme. Preferred enzymes for which the action of the gene encoding such enzyme can be genetically modified include: hexokinase, glucose phosphate isomerase, phosphomannose isomerase (PMI), phosphomannomutase

(PMM), GDP-D-mannose pyrophosphorylase (GMP), GDP-D-mannose:GDP-L-galactose epimerase, GDP-L-galactose phosphorylase, L-galactose-1-P-phosphatase, L-galactose dehydrogenase, and/or L-galactono-γ-lactone dehydrogenase. More preferably, a genetically modified plant or microorganism useful in the present invention has a genetic modification which increases the action of an enzyme selected from the group of GDP-D-mannose:GDP-L-galactose epimerase, GDP-L-galactose phosphorylase, L-galactose-1-P-phosphatase, L-galactose dehydrogenase, and/or L-galactono-γ-lactone dehydrogenase. Even more preferably, a genetically modified plant or microorganism useful in the present invention has a genetic modification which increases the action of GDP-D-mannose:GDP-L-galactose epimerase. These enzymes and the reactions catalyzed by such enzymes are illustrated in Figs. 1A-1C.

Prior to the present invention, without knowing the L-ascorbic acid biosynthetic (i.e., production) pathway, previous mutagenesis and screening efforts were limited in that only non-lethal mutations could be detected. One embodiment of the present invention relates to elimination of a key competing enzyme that diverts carbon flow from L-ascorbic acid synthesis. If such enzyme is absolutely required for growth on glucose, then mutants lacking the enzyme (and, therefore, having increased carbon flow to L-ascorbic acid) would have been nonviable and not have been detected during prior screening efforts. One such enzyme is phosphofructokinase (PFK) (See Fig. 2A). PFK is required for growth on glucose, and is the major step drawing carbon away from L-ascorbic acid biosynthesis (Fig. 2A). Elimination of PFK would render the cells nonviable on glucose-based media. Selection of a conditional mutant where PFK was inactivated by temperature shift, for example, may allow development of a L-ascorbic acid process where cell growth is achieved under permissive fermentation conditions, and L-ascorbic acid production (from glucose) is initiated by a shift to non-permissive condition. In this example, the temperature shift would eliminate carbon flow from glucose to glycolysis via PFK, thereby shunting carbon into the L-ascorbic acid branch of metabolism. This approach has application not only in natural L-ascorbic acid producing organisms, but also in L-ascorbic acid recombinant systems (genetically engineered plant or microorganisms) as discussed herein.

Knowing the identity and mechanism of the rate-limiting pathway enzymes in the L-ascorbic acid production pathway allows for design of specific inhibitors of the enzymes that are also growth inhibitory. Selection of mutants resistant to the inhibitors allows for the isolation of strains that contain L-ascorbic acid-pathway enzymes with more favorable kinetic properties. Therefore, one embodiment of the present invention is to identify inhibitors of the enzymes that are also growth inhibitory. These inhibitors are then used to select genetic mutants that overcome this inhibition and produce L-ascorbic acid at high levels. In this embodiment, the resultant plant or microorganism is a non-recombinant strain which can then be further modified by recombinant technology, if desired. In recombinant L-ascorbic acid producing strains, random mutagenesis and screening can be used as a final step to increase L-ascorbic acid production.

In yet another embodiment genetic modifications are made to an L-ascorbic acid producing organism directly. This allows one to build upon a base of data acquired during prior classical strain improvement efforts, and perhaps more importantly, allows one to take advantage of undefined beneficial mutations that occurred during classical strain improvement. Furthermore, fewer problems are encountered when expressing native, rather than heterologous, genes. The most advanced system for development of genetic systems for microalgae has been developed for *Chlamydomonas reinhardtii*. Preferably, development of such a genetically modified production organism would include: isolation of mutant(s) with a specific nutritional requirement for use with a cloned selectable marker gene (similar to the *ura3* mutants used in yeast and fungal systems); a cloned selectable marker such as *URA3* or alternatively, identification and cloning of a gene that specifies resistance to a toxic compound (this would be analogous to the use of antibiotic resistance genes in bacterial systems, and, as is the case in yeast and other fungi, a means of inserting/removing the marker gene repeatedly would be required, unless several different selectable markers were developed); a transformation system for introducing DNA into the production organism and achieving stable transformation and expression; and, a promoter system (preferably several) for high-level expression of cloned genes in the organism.

Another embodiment of the present invention, discussed in detail below, is to place

organisms (i.e., higher plants and microalgae) into a plant or microorganism that is more amenable to molecular genetic manipulation, including endogenous L-ascorbic acid producing microorganisms and suitable plants. For example, it is possible to identify a suitable non-pathogenic organism based on the requirement of growth (on glucose) at low pH (i.e., acid-tolerant organisms, discussed in detail below).

One suitable candidate for recombinant production in any suitable host organism is the gene (nucleic acid molecule) encoding GDP-D-mannose:GDP-L-galactose epimerase and homologues of the GDP-D-mannose:GDP-L-galactose epimerase, as well as any other epimerase that has structural homology at the primary (i.e., sequence) or tertiary (i.e., three dimensional) level, to a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase, or to a UDP-galactose 4-epimerase. Many microorganisms produce GDP-D-mannose as a precursor to exopolysaccharide and glycoprotein production, even though such organisms may not make L-ascorbic acid. This aspect of the present invention is discussed in detail below.

Referring to Figs. 1A-1C, at least some of the enzymes from glucose-6-phosphate to GDP-D-mannose are present in many organisms. In fact, the entire sequence is present in bacteria such as *Azotobacter vinelandii* and *Pseudomonas aeruginosa*, and make up the early steps in the biosynthesis of the exopolysaccharide alginate. In this regard, it is possible that the only thing preventing these organisms from producing L-ascorbic acid could be the lack of GDP-D-mannose:GDP-L-galactose epimerase. The presence of PMI, PMM and GMP (see Fig. 1A) in so many organisms is important for two reasons. First, these organisms themselves could serve as alternate hosts for L-ascorbic acid production, by building on the existing early pathway enzymes and adding the required cloned genes (the epimerase and possibly others). Second, the genes encoding PMI, PMM and GMP can be cloned into a new organism where, together with the cloned epimerase, they would encode the overall pathway from glucose-6-phosphate to GDP-L- galactose.

In order to screen genomic DNA or cDNA libraries from different organisms and to isolate nucleic acid molecules encoding these enzymes such as the GDP-D-mannose:GDP-L-galactose epimerase, one can use any of a variety of standard molecular and biochemical techniques. For example, the GDP-D-mannose:GDP-L-galactose

acid sequence can be determined (including, if necessary, the sequence of internal peptide fragments), and this information can be used to design degenerate primers for amplifying a gene fragment from the organism's DNA. This fragment would then be used to probe the library, and subsequently fragments that hybridize to the probe would be cloned in that organism or another suitable production organism. There is ample precedent for plant enzymes being expressed in an active form in bacteria, such as *E. coli*. Alternatively, yeast are also a suitable candidate for developing a heterologous system for L-ascorbic acid production.

It is to be understood that the present invention discloses a method comprising the use of a microorganism with an ability to produce commercially useful amounts of L-ascorbic acid in a fermentation process (i.e., preferably an enhanced ability to produce L-ascorbic acid compared to a wild-type microorganism cultured under the same conditions). This method is achieved by the genetic modification of one or more genes encoding a protein involved in an L-ascorbic acid pathway which results in the production (expression) of a protein having an altered (e.g., increased or decreased) function as compared to the corresponding wild-type protein. Preferably, such genetic modification is achieved by recombinant technology. It will be appreciated by those of skill in the art that production of genetically modified plants or microorganisms having a particular altered function as described elsewhere herein (e.g., an enhanced ability to produce GDP-D-mannose:GDP-L-galactose epimerase), such as by transformation of the plant or microorganism with a nucleic acid molecule which encodes a particular enzyme, can produce many organisms meeting the given functional requirement, albeit by virtue of a variety of different genetic modifications. For example, different random nucleotide deletions and/or substitutions in a given nucleic acid sequence may all give rise to the same phenotypic result (e.g., decreased enzymatic activity of the protein encoded by the sequence). The present invention contemplates any such genetic modification which results in the production of a plant or microorganism having the characteristics set forth herein.

A microorganism to be used in the fermentation method of the present invention is preferably a bacterium, a fungus, or a microalga which has been genetically modified according to the disclosure above. More preferably, a microorganism useful in the present

invention is a microalga which is capable of producing L-ascorbic acid, although the present invention includes microorganisms which are genetically engineered to produce L-ascorbic acid using the knowledge of the key components of the pathway and the guidance provided herein. Even more preferably, a microorganism useful in the present invention is an acid-tolerant microorganism, such as microalgae of the genera *Prototheca* and *Chlorella*. Acid-tolerant yeast and bacteria are also known in the art. Acid-tolerant microorganisms are discussed in detail below. Particularly preferred microalgae include microalgae of the genera, *Prototheca* and *Chlorella*, with *Prototheca* being most preferred. All known species of *Prototheca* produce L-ascorbic acid. Production of ascorbic acid by microalgae of the genera *Prototheca* and *Chlorella* is described in detail in U.S. Patent No. 5,792,631, issued August 11, 1998, and in U.S. Patent No. 5,900,370, issued May 4, 1999, both of which are incorporated herein by reference in their entirety. Preferred bacteria for use in the present invention include, but are not limited to, *Azotobacter*, *Pseudomonas*, and *Escherichia*, although acid-tolerant bacteria are more preferred. Preferred fungi for use in the present invention include yeast, and more preferably, yeast of the genus, *Saccharomyces*. A microorganism for use in the fermentation method of the present invention can also be referred to as a production organism. According to the present invention, microalgae can be referred to herein either as microorganisms or as plants.

A preferred plant to genetically modify according to the present invention is preferably a plant suitable for consumption by animals, including humans. More preferably, such a plant is a plant that naturally produces L-ascorbic acid, although other plants can be genetically modified to produce L-ascorbic acid using the guidance provided herein.

The L-ascorbic acid production pathways of the microalgae *Prototheca* and *Chlorella pyrenoidosa* will be addressed as specific embodiments of the present invention are described below. It will be appreciated that other plants and, in particular, other microorganisms, have similar L-ascorbic acid pathways and genes and proteins having similar structure and function within such pathways. It will also be appreciated that plants and microorganisms which do not naturally produce L-ascorbic acid can be modified according to the present invention to produce L-ascorbic acid. As such, the principles

discussed below with regard to *Prototheca* and *Chlorella pyrenoidosa* are applicable to other plants and microorganisms, including genetically modified plants and microorganisms.

In one embodiment of the present invention, the action of an enzyme in the L-ascorbic acid production pathway is increased by amplification of the expression (i.e., overexpression) of an enzyme in the pathway, and particularly, the GDP-D-mannose:GDP-L-galactose epimerase, homologues of the epimerase, and/or enzymes downstream of the epimerase. Overexpression of an enzyme can be accomplished, for example, by introduction of a recombinant nucleic acid molecule encoding the enzyme. It is preferred that the gene encoding an enzyme in the L-ascorbic acid production pathway be cloned under control of an artificial promoter. The promoter can be any suitable promoter that will provide a level of enzyme expression required to maintain a sufficient level of L-ascorbic acid in the production organism. Preferred promoters are constitutive (rather than inducible) promoters, since the need for addition of expensive inducers is therefore obviated. The gene dosage (copy number) of a recombinant nucleic acid molecule according to the present invention can be varied according to the requirements for maximum product formation. In one embodiment, the recombinant nucleic acid molecule encoding a gene in the L-ascorbic acid production pathway is integrated into the chromosomes of the microorganism.

It is another embodiment of the present invention to provide a microorganism having one or more enzymes in the L-ascorbic acid production pathway with improved affinity for its substrates. An enzyme with improved affinity for its substrates can be produced by any suitable method of genetic modification or protein engineering. For example, computer-based protein engineering can be used to design an epimerase protein with greater stability and better affinity for its substrate. See for example, Maulik et al., 1997, *Molecular Biotechnology: Therapeutic Applications and Strategies*, Wiley-Liss, Inc., which is incorporated herein by reference in its entirety.

Recombinant nucleic acid molecules encoding proteins in the L-ascorbic acid production pathway can be modified to enhance or reduce the function (i.e., activity) of the protein, as desired to increase L-ascorbic acid production, by any suitable method of genetic modification. For example, a recombinant nucleic acid molecule encoding an

enzyme can be modified by any method for inserting, deleting, and/or substituting nucleotides, such as by error-prone PCR. In this method, the gene is amplified under conditions that lead to a high frequency of misincorporation errors by the DNA polymerase used for the amplification. As a result, a high frequency of mutations are obtained in the PCR products. The resulting gene mutants can then be screened for enhanced substrate affinity, enhanced enzymatic activity, or reduced/increased inhibitory ability by testing the mutant genes for the ability to confer increased L-ascorbic acid production onto a test microorganism, as compared to a microorganism carrying the non-mutated recombinant nucleic acid molecule.

Another embodiment of the present invention includes a microorganism in which competitive side reactions are blocked, including all reactions for which GDP-D-mannose is a substrate other than the production of L-ascorbic acid. In a preferred embodiment, a microorganism having complete or partial inactivation (decrease in the action of) of genes encoding enzymes which compete with the GDP-D-mannose:GDP-L-galactose epimerase for the GDP-D-mannose substrate is provided. Such enzymes include GDP-D-mannase and/or GDP-D-mannose-dehydrogenase. As used herein, inactivation of a gene can refer to any modification of a gene which results in a decrease in the activity (i.e., expression or function) of such a gene, including attenuation of activity or complete deletion of activity.

As discussed above, a particularly preferred aspect of the method to produce L-ascorbic acid by fermentation of a genetically modified microorganism of the present invention includes the step of culturing in a fermentation medium a microorganism having a genetic modification to increase the action of an epimerase that catalyzes conversion of GDP-D-mannose to GDP-L-galactose. According to the present invention, such an epimerase can include the endogenous GDP-D-mannose:GDP-L-galactose epimerase from the L-ascorbic acid pathway, described above, as well as any other epimerase that has structural homology at the primary (i.e., sequence) or tertiary (i.e., three dimensional) level, to a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase, or to a UDP-galactose 4-epimerase. Such structural homology is discussed in detail below. Preferably, such an epimerase is capable of catalyzing the conversion of GDP-D-mannose to GDP-L-galactose. In one embodiment, the genetic modification includes transformation of the

microorganism with a recombinant nucleic acid molecule that expresses such an epimerase.

Therefore, the epimerase encompassed in the method and organisms of the present invention includes the endogenous epimerase which operates in the naturally occurring ascorbic acid biosynthetic pathway (referred to herein as GDP-D-mannose:GDP-L-galactose epimerase), GDP-4-keto-6-deoxy-D-mannose epimerase/ reductases, and any other epimerase which is capable of catalyzing the conversion of GDP-D mannose to GDP-L-galactose and which is structurally homologous to a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase or a UDP-galactose 4-epimerase. An epimerase that catalyzes conversion of GDP-D-mannose to GDP-L-galactose according the present invention can be identified by biochemical and functional characteristics as well as structural characteristics. For example, an epimerase according to the present invention is capable of acting on GDP-D-mannose as a substrate, and more particularly, such an epimerase is capable of catalyzing the conversion of GDP-D-mannose to GDP-L-galactose. It is to be understood that such capabilities need not necessarily be the normal or natural function of the epimerase as it acts in its endogenous (i.e., natural) environment. For example, GDP-4-keto-6-deoxy-D-mannose epimerase/reductase in its natural environment under normal conditions, catalyzes the conversion of GDP-D-mannose to GDP-L-fucose and does not act directly on GDP-D-mannose (See Fig.8A, B), however, such an epimerase is encompassed by the present invention for use in catalyzing the conversion of GDP-D-mannose to GDP-L-galactose for production of ascorbic acid, to the extent that it is capable of, or can be modified to be capable of, catalyzing the conversion of GDP-D-mannose to GDP-L-galactose. Therefore, the present invention includes epimerases which have the desired enzyme activity for use in production of ascorbic acid, are capable of having such desired enzyme activity, and/or are capable of being modified or induced to have such desired enzyme activity.

In one embodiment, an epimerase according to the present invention includes an epimerase that catalyzes the reaction depicted in Fig. 7. In another embodiment, an epimerase according to the present invention includes an epimerase that catalyzes the first of the reactions depicted in Fig. 8B. In one embodiment, an epimerase according to the

present invention binds to NADPH. In another embodiment, an epimerase according to the present invention is NADPH-dependent for enzyme activity.

As discussed above, the present inventors have discovered that a key enzyme in L-ascorbic acid biosynthesis in plants and microorganisms is GDP-D-mannose:GDP-L-galactose epimerase (refer to Figs. 1A-1C). One embodiment of the invention described herein is directed to the manipulation of this enzyme and structural homologues of this enzyme to increase L-ascorbic acid production in genetically engineered plants and/or microorganisms. More particularly, the GDP-D-mannose:GDP-L-galactose epimerase of the L-ascorbic acid pathway and GDP-4-keto-6-deoxy-D-mannose epimerase/reductases are believed to be structurally homologous at both the sequence and tertiary structure level; a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase is believed to be capable of functioning in the L-ascorbic acid biosynthetic pathway; and a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase or homologue thereof may be superior to a GDP-D-mannose-GDP-L-galactose epimerase for increasing L-ascorbic acid production in genetically engineered plants and/or microorganisms. Furthermore, the present inventors disclose the use of a nucleotide sequence encoding all or part of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase as a probe to identify the gene encoding GDP-D-mannose:GDP-L-galactose epimerase. Similarly, the present inventors disclose the use of a nucleotide sequence of the gene encoding GDP-4-keto-6-deoxy-D-mannose epimerase/reductase to design oligonucleotide primers for use in a PCR-based strategy for identifying and cloning a gene encoding GDP-D-mannose:GDP-L-galactose epimerase.

Without being bound by theory, the present inventors believe that the following evidence supports the novel concept that the GDP-D-mannose:GDP-L-galactose epimerase and GDP-4-keto-6-deoxy-D-mannose epimerase/reductases have significant structural homology at the level of sequence and/or tertiary structure, and that the GDP-4-keto-6-deoxy-D-mannose epimerase/reductases and/or homologues thereof would be useful for production of ascorbic acid and/or for isolating the endogenous GDP-D-mannose:GDP-L-galactose epimerase.

Although prior to the present invention, it was not known that the GDP-D-mannose:GDP-L-galactose epimerase enzyme (also known as GDP-D-mannose-3,5-epimerase) plays a critical role in L-ascorbic acid biosynthesis, this enzyme was previously

described to catalyze the overall reversible reaction between GDP-D-mannose and GDP-L-galactose (Barber, 1971, *Arch. Biochem. Biophys.* 147:619-623; Barber, 1975, *Arch. Biochem. Biophys.* 167:718-722; Barber, 1979, *J. Biol. Chem.* 254:7600-7603; Hebda, et al., 1979, *Arch. Biochem. Biophys.* 194:496-502; Barber and Hebda, 1982, *Meth. Enzymol.*, 83:522-525). Despite these studies, GDP-D-mannose:GDP-L-galactose epimerase has never been well characterized nor has the gene encoding this enzyme been cloned and sequenced. Since the original work by Barber, GDP-D-mannose:GDP-L-galactose epimerase activity has been detected in the colorless microalga *Prototheca moriformis* by the assignee of the present application, and in *Arabidopsis thaliana* and pea embryonic axes (Wheeler, et al., 1998, *ibid.*).

Barber (1979, *J. Biol. Chem.* 254:7600-7603) proposed a mechanism for GDP-D-mannose:GDP-L-galactose epimerase partially purified from the green microalga *Chlorella pyrenoidosa*. The overall conversion of GDP-D-mannose to GDP-L-galactose was proposed to proceed by oxidation of the hexosyl moiety at C-4 to a keto intermediate, ene-diol formation, and inversion of the configurations at C-3 and C-5 upon rehydration of the double bonds and stereospecific reduction of the keto group. The proposed mechanism is depicted in Fig. 7.

Based on Barber's work, Feingold and Avigad (1980, In *The Biochemistry of Plants*, Vol. 3: Carbohydrates; Structure and Function, P.K. Stompf and E.E. Conn, eds., Academic Press, NY) elaborated further on the proposed mechanism for GDP-D-mannose:GDP-L-galactose epimerase. This mechanism is based on the assumption that the epimerase contains tightly bound NAD^+, and transfer of a hydride ion from C-4 of the substrate (GDP-D-mannose) to enzyme-associated NAD^+ converts the enzyme to the reduced (NADH)form, generating enzyme-bound GDP-4-keto-D-mannose. The latter would then undergo epimerization by an ene-diol mechanism. The final product (GDP-L-galactose) would be released from the enzyme after stereospecific transfer of the hydride ion originally removed from C-4, simultaneously regenerating the oxidized form of the enzyme.

L-fucose (6-deoxy-L-galactose) is a component of bacterial lipopolysaccharides, mammalian and plant glycoproteins and polysaccharides of plant cell walls. L-fucose is synthesized *de novo* from GDP-D-mannose by the sequential action of GDP-D-mannose-

4,6-dehydratase (an NAD(P)-dependent enzyme), and a bifunctional GDP-4-keto-6-deoxy-D-mannose epimerase/reductase (NADPH-dependent), also referred to in scientific literature as GDP-fucose synthetase (Rizzi, et al., 1998, *Structure* 6:1453-1465; Somers, et al., 1998, *Structure* 6:1601-1612). This pathway for L-fucose biosynthesis appears to be ubiquitous (Rizzi, et al., 1998, *Structure* 6:1453-1465). The mechanisms for GDP-D-mannose-4,6-dehydratase and GDP-4-keto-6-deoxy-D-mannose epimerase/reductase are shown in Fig. 8A, B (Chang, et al., 1988, *J. Biol. Chem.* 263:1693-1697; Barber, 1980, *Plant Physiol.* 66:326-329).

Comparison of Figs. 7 and 8A, B reveals that Barber's proposed mechanism for GDP-D-mannose:GDP-L-galactose epimerase is analogous to the reaction mechanism for GDP-4-keto-6-deoxy-D-mannose epimerase/reductase. The same mechanism has also been demonstrated for the epimerization reaction that occurs in the biosynthesis of two TDP-6-deoxy hexoses, TDP-L-rhamnose and TDP-6-deoxy-L-talose, from TDP-D-glucose (Liu and Thorson, 1994, *Ann. Rev. Microbiol.* 48:223-256). In the latter cases, however, the final reduction at C-4 is catalyzed by NADPH-dependent reductases that are separate from the epimerase enzyme. These reductases have opposite stereospecificity, providing either TDP-L-rhamnose or TDP-6-deoxy-L-talose (Liu and Thorson, 1994, *Ann. Rev. Microbiol.* 48:223-256).

In all of the mechanisms described above, NAD(P)H is required for the final reduction at C-4 (refer to Fig. 8B). In the work of Hebda, et al. (1979, *Arch. Biochem. Biophys.* 194:496-502), it was reported that GDP-D-mannose:GDP-L-galactose epimerase from *C. pyrenoidosa* did not require NAD, NADP or NADH for activity. Strangely, NADPH was not tested. Based on the analogous mechanisms shown in Figs. 7 and 8A, B, the present inventors believe that it is likely that GDP-D-mannose:GDP-L-galactose epimerase from *C. pyrenoidosa* requires NADPH for the final reduction step. Why activity was detected *in vitro* without NADPH addition is not known, but tight *binding of NADPH to the enzyme could explain this observation. On the other hand, if the proposed mechanism of Feingold and Avigad (1980, in *The Biochemistry of Plants*, Vol. 3, p. 101-170: Carbohydrates; Structure and Function, P.K. Stompf and E.E. Conn, ed., Academic Press, NY) is correct, the reduced enzyme-bound cofactor generated in the first oxidation step of the epimerase reaction would serve as the source of electrons for

the final reduction of the keto group at C-4 back to the alcohol. Thus no addition of exogenous reduced cofactor would be required for activity *in vitro*.

Recently, a human gene encoding the bifunctional GDP-4-keto-6-deoxy-D-mannose epimerase/reductase was cloned and sequenced (Tonetti, et al., 1996, *J. Biol. Chem.* 271-27274-27279). This amino acid sequence of the human GDP-4-keto-6-deoxy-D-mannose epimerase/reductase shows significant homology (29% identity) to the *E. coli* GDP-4-keto-6-deoxy-D-mannose epimerase/reductase (Tonetti, et al., 1998, *Acta Cryst.* D54:684-686; Somers, et al., 1998, *Structure* 6:1601-1612, both of which are incorporated herein by reference in their entireties). Tonetti et al. and Somers et al. additionally disclosed the tertiary (three dimensional) structure of the *E. coli* GDP-4-keto-6-deoxy-D-mannose epimerase/reductase (also known as GDP-fucose synthetase), and noted significant structural homology with another epimerase, UDP-galactose 4-epimerase (GalE). These epimerases also share significant homology at the sequence level. Since no gene encoding a GDP-D-mannose:GDP-L-galactose epimerase has been cloned and sequenced, homology with genes encoding GDP-4-keto-6-deoxy-D-mannose epimerase/reductases or with genes encoding a UDP-galactose 4-epimerase has not been demonstrated. However, based on the similarity of the reaction products for GDP-D-mannose:GDP-L-galactose epimerase and GDP-4-keto-6-deoxy-D-mannose epimerase/reductase (i.e., GDP-L-galactose and GDP-6-deoxy-L-galactose [i.e., GDP-L-fucose], respectively) and the common catalytic mechanisms (Figs. 7 and 8A, B) the present inventors believe that the genes encoding the enzymes will have a high degree of sequence homology, as well as tertiary structural homology.

Significant structural homology between GDP-D-mannose:GDP-L-galactose epimerase and GDP-4-keto-6-deoxy-D-mannose epimerase/reductases may allow a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase, or a homologue thereof, to function in the L-ascorbic acid biosynthetic pathway, and a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase could potentially be even better than a GDP-D-mannose-GDP-L-galactose epimerase for increasing L-ascorbic acid production in genetically engineered plants and/or microorganisms. Furthermore, a nucleotide sequence encoding all or part of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase can be used as a probe to identify the gene encoding GDP-D-mannose:GDP-L-galactose epimerase. Likewise, the

nucleotide sequence of the gene encoding GDP-4-keto-6-deoxy-D-mannose epimerase/ reductase can be used to design oligonucleotide primers for use in a PCR-based strategy for identifying and cloning a gene encoding GDP-D-mannose:GDP-L-galactose epimerase.

The ability to substitute GDP-4-keto-6-D-mannose epimerase/reductase for GDP-D-mannose:GDP-L-galactose epimerase to enhance L-ascorbic acid biosynthesis in plants or microorganisms depends on the ability of GDP-4-keto-6-deoxy-D-mannose epimerase/ reductase to act directly on GDP-D-mannose to form GDP-L-galactose. Evidence supporting this possibility already exists. *Arabidopsis thaliana murl* mutants are defective in GDP-D-mannose-4,6-dehydratase activity (Bonin, et al., 1997, *Proc. Natl. Acad. Sci.* 94:2085-2090). These mutants are thus blocked in GDP-L-fucose biosynthesis, and consequently have less than 2% of the normal amounts of L-fucose in the primary cell walls of aerial portions of the plant (Zablackis, et al., 1996, *Science* 272:1808-1810). The *murl* mutants are more brittle than wild-type plants, are slightly dwarfed and have an apparently normal life cycle (Zablackis, et al., 272:1808-1810). When *murl* mutants are grown in the presence of exogenous L-fucose, the L-fucose content in the plant is restored to the wild-type state (Bonin, et al., 1997, *Proc. Natl. Acad. Sci.* 94:2085-2090). It was discovered (Zablackis, et al., 1996, *Science* 272:1808-1810) that *murl* mutants contain, in the hemicellulose xyloglucan component of the primary cell wall, L-galactose in place of the normal L-fucose. L-galactose is not normally found in the xyloglucan component, but in *murl* mutants L-galactose partly replaces the terminal L-fucosyl residue. Bonin, et al. (1997, *Proc. Natl. Acad. Sci.* 94:2085-2090) hypothesized that in the absence of a functional GDP-D-mannose-4,6-dehydratase in the *murl* mutants, the GDP-4-keto-6-deoxy-D-mannose epimerase/reductase normally involved in L-fucose synthesis may be able to use GDP-D-mannose directly, forming GDP-L-galactose. Another possibility, however, is that the enzymes involved in L-ascorbic acid biosynthesis in *A. thaliana* are responsible for forming GDP-L-galactose in the *murl* mutant. If this were true, it would suggest that in the wild-type plant, some mechanism exists that prevents GDP-L-galactose formed in the L-ascorbic acid pathway from entering cell wall biosynthesis and substituting for (competing with) GDP-L-fucose for incorporation into the xyloglucan

component (since L-galactose is not present in the primary cell wall of the wild-type plant).

Because of the similar reaction mechanisms of GDP-D-mannose:GDP-L-galactose epimerase and GDP-4-keto-6-deoxy-D-mannose epimerase/reductase, and because of the evidence that GDP-4-keto-6-deoxy-D-mannose epimerase/reductase can act directly on GDP-D-mannose to form GDP-L-galactose, the present inventors believe that genes encoding all epimerases and epimerase/reductases that act on GDP-D-mannose have high homology. As such, one aspect of the present invention relates to the use of any epimerase (and nucleic acid sequences encoding such epimerase) having significant homology (at the primary, secondary and/or tertiary structure level) to a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase or to a UDP-galactose 4-epimerase for the purpose of improving the biosynthetic production of L-ascorbic acid.

Therefore, as described above, one embodiment of the present invention relates to a method for producing ascorbic acid or esters thereof in a microorganism, which includes culturing a microorganism having a genetic modification to increase the action of an epimerase that catalyzes conversion of GDP-D-mannose to GDP-L-galactose. Also included in the present invention are genetically modified microorganisms and plants in which the genetic modification increases the action of an epimerase that catalyzes conversion of GDP-D-mannose to GDP-L-galactose.

According to the present invention, an increase in the action of the GDP-D-mannose:GDP-L-galactose epimerase in the L-ascorbic acid production pathway can be achieved by genetic modifications which include, but are not limited to overexpression of the GDP-D-mannose:GDP-L-galactose epimerase gene, a homologue of such gene, or of any recombinant nucleic acid sequence encoding an epimerase that is homologous in primary (nucleic acid or amino acid sequence) or tertiary (three dimensional protein) structure to a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase or a UDP-galactose 4-epimerase, such as by overexpression of a recombinant nucleic acid molecule encoding the epimerase gene or a homologue thereof, and/or by mutation of the endogenous or recombinant gene to enhance expression of the gene.

According to the present invention, an epimerase that has a tertiary structure that is homologous to the tertiary structure of a GDP-4-keto-6-deoxy-D-mannose epimerase/

reductase is an epimerase that has a tertiary structure that substantially conforms to the tertiary structure of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by the atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws (Table 12). In another embodiment, an epimerase that has a tertiary structure that is homologous to the tertiary structure of a GDP-4-keto-6-deoxy-D-mannose epimerase/ reductase is an epimerase that has a tertiary structure that substantially conforms to the tertiary structure of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by the atomic coordinates having Brookhaven Protein Data Bank Accession Code 1GFS. As used herein, a "tertiary structure" or "three dimensional structure" of a protein, such terms being interchangeable, refers to the components and the manner of arrangement of the components in three dimensional space to constitute the protein. The use of the term "substantially conforms" refers to at least a portion of a tertiary structure of an epimerase which is sufficiently spatially similar to at least a portion of a specified three dimensional configuration of a particular set of atomic coordinates (e.g., those represented by Brookhaven Protein Data Bank Accession Code 1bws) to allow the tertiary structure of at least said portion of the epimerase to be modeled or calculated (i.e., by molecular replacement) using the particular set of atomic coordinates as a basis for estimating the atomic coordinates defining the three dimensional configuration of the epimerase.

More particularly, a tertiary structure that substantially conforms to a given set of atomic coordinates is a structure having an average root-mean-square deviation (RMSD) of less than about 2.5 Å, and more preferably, less than about 2 Å, and, in increasing preference, less than about 1.5 Å, less than about 1 Å, less than about 0.5 Å, and most preferably, less than about 0.3 Å, over at least about 25% of the Cα positions as compared to the tertiary structure of a GDP-4-keto-6-deoxy-D-mannose epimerase/ reductase represented by the atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws. In other embodiments, a structure that substantially conforms to a given set of atomic coordinates is a structure wherein such structure has the recited average root-mean-square deviation (RMSD) value over at least about 50% of the Cα positions as compared to the tertiary structure of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by the atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws, and in another embodiment, such structure has the

recited average root-mean-square deviation (RMSD) value over at least about 75% of the Cα positions as compared to the tertiary structure of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by the atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws, and in another embodiment, such structure has the recited average root-mean-square deviation (RMSD) value over about 100% of the Cα positions as compared to the tertiary structure of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by the atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws. Methods to calculate RMSD values are well known in the art. Various software programs for determining the tertiary structural homology between one or more proteins are known in the art and are publicly available, such as QUANTA (Molecular Simulations Inc.).

A preferred epimerase that catalyzes conversion of GDP-D-mannose to GDP-L-galactose according to the method and genetically modified organisms of the present invention includes an epimerase that comprises a substrate binding site having a tertiary structure that substantially conforms to the tertiary structure of the substrate binding site of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by the atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws. Preferably, the tertiary structure of the substrate binding site of the epimerase has an average root-mean-square deviation (RMSD) of less than about 2.5 Å, and more preferably, less than about 2 Å, and, in increasing preference, less than about 1.5 Å, less than about 1 Å, less than about 0.5 Å, and most preferably, less than about 0.3 Å, over at least about 25% of the Cα positions as compared to the tertiary structure of the substrate binding site of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by the atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws. In other embodiments, the tertiary structure of the substrate binding site of the epimerase has the recited average root-mean-square deviation (RMSD) value over at least about 50% of the Cα positions as compared to the tertiary structure of the substrate binding site of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by the atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws, and in another embodiment, the tertiary structure of the substrate binding site of the epimerase has the recited average root-mean-square deviation (RMSD) value over at least about 75% of the Cα positions

as compared to the tertiary structure of the substrate binding site of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by the atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws, and in another embodiment, the tertiary structure of the substrate binding site of the epimerase has the recited average root-mean-square deviation (RMSD) value over about 100% of the Cα positions as compared to the tertiary structure of the substrate binding site of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by the atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws. The tertiary structure of the substrate binding site of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by the atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws is discussed in detail in Rizzi et al., 1998, *ibid.* Additionally, the tertiary structure of the substrate binding site of a GDP-4-keto-6-deoxy-D-mannose epimerase/ reductase represented by the atomic coordinates having Brookhaven Protein Data Bank Accession Code 1GFS is discussed in detail in Somers et al., 1998, *ibid.*

Another preferred epimerase according to the present invention includes an epimerase that comprises a catalytic site having a tertiary structure that substantially conforms to the tertiary structure of the catalytic site of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by the atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws. Preferably, the tertiary structure of the catalytic site of the epimerase has an average root-mean-square deviation (RMSD) of less than about 2.5 Å, and more preferably, less than about 2 Å, and, in increasing preference, less than about 1.5 Å, less than about 1 Å, less than about 0.5 Å, and most preferably, less than about 0.3 Å, over at least about 25% of the Cα positions as compared to the tertiary structure of the catalytic site of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by the atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws. In other embodiments, the tertiary structure of the catalytic site of the epimerase has the recited average root-mean-square deviation (RMSD) value over at least about 50% of the Cα positions as compared to the tertiary structure of the catalytic site of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by the atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws, and in another embodiment, the tertiary structure of the catalytic site of the epimerase has the recited

average root-mean-square deviation (RMSD) value over at least about 75% of the Cα positions as compared to the tertiary structure of the catalytic site of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by the atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws, and in another embodiment, the tertiary structure of the catalytic site of the epimerase has the recited average root-mean-square deviation (RMSD) value over 100% of the Cα positions as compared to the tertiary structure of the catalytic site of a GDP-4-keto-6-deoxy-D-mannose epimerase/ reductase represented by the atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws.

In one embodiment, an epimerase encompassed by the present invention includes an epimerase that has a catalytic site which includes amino acid residues: serine, tyrosine and lysine. In a preferred embodiment, the tertiary structure positions of the amino acid residues serine, tyrosine and lysine substantially conform to the tertiary structure position of residues Ser107, Tyr136 and Lys140, respectively, as represented by atomic coordinates in Brookhaven Protein Data Bank Accession Code 1bws. The tertiary structure of the catalytic site of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by the atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws is discussed in detail in Rizzi et al., 1998, *ibid.* Additionally, the tertiary structure of the catalytic site of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by the atomic coordinates having Brookhaven Protein Data Bank Accession Code 1GFS is discussed in detail in Somers et al., 1998, *ibid.*

In an even more preferred embodiment, the above definition of "substantially conforms" can be further defined to include atoms of amino acid side chains. As used herein, the phrase "common amino acid side chains" refers to amino acid side chains that are common to both the structures which substantially conforms to a given set of atomic coordinates and the structure that is actually represented by such atomic coordinates. Preferably, a tertiary structure that substantially conforms to a given set of atomic coordinates is a structure having an average root-mean-square deviation (RMSD) of less than about 2.5 Å, and more preferably, less than about 2 Å, and, in increasing preference, less than about 1.5 Å, less than about 1 Å, less than about 0.5 Å, and most preferably, less than about 0.3 Å over at least about 25% of the common amino acid side chains as

compared to the tertiary structure represented by the given set of atomic coordinates. In another embodiment, a structure that substantially conforms to a given set of atomic coordinates is a structure having the recited average root-mean-square deviation (RMSD) value over at least about 50% of the common amino acid side chains as compared to the tertiary structure represented by the given set of atomic coordinates, and in another embodiment, such structure has the recited average root-mean-square deviation (RMSD) value over at least about 75% of the common amino acid side chains as compared to the tertiary structure represented by the given set of atomic coordinates, and in another embodiment, such a structure has the recited average root-mean-square deviation (RMSD) value over 100% of the common amino acid side chains as compared to the tertiary structure represented by the given set of atomic coordinates.

A tertiary structure of an epimerase which substantially conforms to a specified set of atomic coordinates can be modeled by a suitable modeling computer program such as MODELER (A. Sali and T.L. Blundell, *J. Mol. Biol.*, vol. 234:779-815, 1993 as implemented in the Insight II Homology software package (Insight II (97.0), MSI, San Diego)), using information, for example, derived from the following data: (1) the amino acid sequence of the epimerase; (2) the amino acid sequence of the related portion(s) of the protein represented by the specified set of atomic coordinates having a three dimensional configuration; and, (3) the atomic coordinates of the specified three dimensional configuration. Alternatively, a tertiary structure of an epimerase which substantially conforms to a specified set of atomic coordinates can be modeled using data generated from analysis of a crystallized structure of the epimerase. A tertiary structure of an epimerase which substantially conforms to a specified set of atomic coordinates can also be calculated by a method such as molecular replacement. Methods of molecular replacement are generally known by those of skill in the art (generally described in Brunger, *Meth. Enzym.*, vol. 276, pp. 558-580, 1997; Navaza and Saludjian, *Meth. Enzym.*, vol. 276, pp. 581-594, 1997; Tong and Rossmann, *Meth. Enzym.*, vol. 276, pp. 594-611, 1997; and Bentley, *Meth. Enzym.*, vol. 276, pp. 611-619, 1997, each of which are incorporated by this reference herein in their entirety) and are performed in a software program including, for example, XPLOR (Brunger, et al., *Science*, vol. 235, p. 458, 1987). In addition, a structure can be modeled using techniques generally described by,

for example, Sali, *Current Opinions in Biotechnology*, vol. 6, pp. 437-451, 1995, and algorithms can be implemented in program packages such as Homology 95.0 (in the program Insight II, available from Biosym/MSI, San Diego, CA). Use of Homology 95.0 requires an alignment of an amino acid sequence of a known structure having a known three dimensional structure with an amino acid sequence of a target structure to be modeled. The alignment can be a pairwise alignment or a multiple sequence alignment including other related sequences (for example, using the method generally described by Rost, *Meth. Enzymol.*, vol. 266, pp. 525-539, 1996) to improve accuracy. Structurally conserved regions can be identified by comparing related structural features, or by examining the degree of sequence homology between the known structure and the target structure. Certain coordinates for the target structure are assigned using known structures from the known structure. Coordinates for other regions of the target structure can be generated from fragments obtained from known structures such as those found in the Protein Data Bank maintained by Brookhaven National Laboratory, Upton, NY. Conformation of side chains of the target structure can be assigned with reference to what is sterically allowable and using a library of rotamers and their frequency of occurrence (as generally described in Ponder and Richards, *J. Mol. Biol.*, vol. 193, pp. 775-791, 1987). The resulting model of the target structure, can be refined by molecular mechanics (such as embodied in the program Discover, available from Biosym/MSI) to ensure that the model is chemically and conformationally reasonable.

According to the present invention, an epimerase that has a nucleic acid sequence that is homologous at the primary structure level (i.e., is a homologue of) to a nucleic acid sequence encoding a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase or a UDP-galactose 4-epimerase includes any epimerase encoded by a nucleic acid sequence that is at least about 15%, and preferably at least about 20%, and more preferably at least about 25%, and even more preferably, at least about 30% identical to a nucleic acid sequence encoding a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase or a UDP-galactose 4-epimerase, and preferably to a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7 or SEQ ID NO:9. Similarly, an epimerase that has an amino acid sequence that is homologous to an amino acid sequence of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase or a UDP-

galactose 4-epimerase includes any epimerase having an amino acid sequence that is at least about 15%, and preferably at least about 20%, and more preferably at least about 25%, and even more preferably, at least about 30% identical to an amino acid sequence of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase or a UDP-galactose 4-epimerase, and preferably to an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8 or SEQ ID NO:10.

According to one embodiment of the present invention, homology or percent identity between two or more nucleic acid or amino acid sequences is performed using methods known in the art for aligning and/or calculating percentage identity. To compare the homology/percent identity between two or more sequences as set forth above, for example, a module contained within DNASTAR (DNASTAR, Inc., Madison, Wisconsin) can be used. In particular, to calculate the percent identity between two nucleic acid or amino acid sequences, the Lipman-Pearson method, provided by the MegAlign module within the DNASTAR program, is preferably used, with the following parameters, also referred to herein as the Lipman-Pearson standard default parameters:

(1) Ktuple = 2;

(2) Gap penalty = 4;

(3) Gap length penalty = 12.

Using the Lipman-Pearson method with these parameters, for example, the percent identity between the amino acid sequence for *E. coli* GDP-4-keto-6-deoxy-D-mannose epimerase/reductase (SEQ ID NO:4) and human GDP-4-keto-6-deoxy-D-mannose epimerase/reductase (FX) (SEQ ID NO:6) is 27.7%, which is comparable to the 27% identity described for these enzymes in Tonetti et al., 1998, *Acta Cryst.* D54:684-686.

According to another embodiment of the present invention, to align two or more nucleic acid or amino acid sequences, for example to generate a consensus sequence or evaluate the similarity at various positions between such sequences, a CLUSTAL alignment program (e.g., CLUSTAL, CLUSTAL V, CLUSTAL W), also available as a module within the DNASTAR program, can be used using the following parameters, also referred to herein as the CLUSTAL standard default parameters:

<u>Multiple Alignment Parameters (i.e., for more than 2 sequences)</u>:

(1) Gap penalty = 10;

(2) Gap length penalty = 10;

Pairwise Alignment Parameters (i.e., for two sequences):

(1) Ktuple = 1;

(2) Gap penalty = 3;

(3) Window = 5;

(4) Diagonals saved = 5.

According to the present invention, a GDP-4-keto-6-deoxy-D-mannose epimerase/ reductase can be a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase from any organism, including *Arabidopsis thaliana*, *Escherichia coli*, and human. A nucleic acid sequence encoding a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase from *Arabidopsis thaliana* is represented herein by SEQ ID NO:1. SEQ ID NO:1 encodes a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase having an amino acid sequence represented herein as SEQ ID NO:2. A nucleic acid sequence encoding a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase from *Escherichia coli* is represented herein by SEQ ID NO:3. SEQ ID NO:3 encodes a GDP-4-keto-6-deoxy-D-mannose epimerase/ reductase having an amino acid sequence represented herein as SEQ ID NO:4. A nucleic acid sequence encoding a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase from *homo sapiens* is represented herein by SEQ ID NO:5. SEQ ID NO:5 encodes a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase having an amino acid sequence represented herein as SEQ ID NO:6.

According to the present invention, a UDP-galactose 4-epimerase can be a UDP-galactose 4-epimerase from any organism, including *Escherichia coli* and human. A nucleic acid sequence encoding a UDP-galactose 4-epimerase from *Escherichia coli* is represented herein by SEQ ID NO:7. SEQ ID NO:7 encodes a UDP-galactose 4-epimerase having an amino acid sequence represented herein as SEQ ID NO:8. A nucleic acid sequence encoding a UDP-galactose 4-epimerase from *homo sapiens* is represented herein by SEQ ID NO:9. SEQ ID NO:9 encodes a UDP-galactose 4-epimerase having an amino acid sequence represented herein as SEQ ID NO:10.

In a preferred embodiment, an epimerase encompassed by the present invention has an amino acid sequence that aligns with the amino acid sequence of SEQ ID NO:11, for example using a CLUSTAL alignment program, wherein amino acid residues in the

amino acid sequence of the epimerase align with 100% identity with at least about 50% of non-Xaa residues in SEQ ID NO:11, and preferably at least about 75% of non-Xaa residues in SEQ ID NO:11, and more preferably, at least about 90% of non-Xaa residues in SEQ ID NO:11, and even more preferably 100% of non-Xaa residues in SEQ ID NO:11. The percent identity of residues aligning with 100% identity with non-Xaa residues can be simply calculated by dividing the number of 100% identical matches at non-Xaa residues in SEQ ID NO:11 by the total number of non-Xaa residues in SEQ ID NO:11. A preferred nucleic acid sequence encoding an epimerase encompassed by the present invention include a nucleic acid sequence encoding an epimerase having an amino acid sequence with the above described identity to SEQ ID NO:11. Such an alignment using a CLUSTAL alignment program is based on the same parameters as previously disclosed herein. SEQ ID NO:11 represents a consensus amino acid sequence of an epimerase which was derived by aligning at least portions of amino acid sequences SEQ ID NO:4, SEQ ID NO:6 and SEQ ID NO:8, as described in Somers et al., 1998, *Structure* 6:1601-1612, and can be approximately duplicated using CLUSTAL.

In another embodiment, an epimerase encompassed by the present invention includes an epimerase that has a catalytic site which includes amino acid residues: serine, tyrosine and lysine. Preferably, such serine, tyrosine and lysine residues are located at positions in the epimerase amino acid sequence which align using a CLUSTAL alignment program with positions Ser105, Tyr134 and Lys138 of consensus sequence SEQ ID NO:11, with positions Ser109, Tyr138 and Lys142 of sequence SEQ ID NO:2, with positions Ser107, Tyr136 and Lys140 of SEQ ID NO:4, with positions Ser114, Tyr143 and Lys147 of sequence SEQ ID NO:6, with positions Ser124, Tyr149 and Lys153 of sequence SEQ ID NO:8 or with positions Ser132, Tyr157 and Lys161 of sequence SEQ ID NO:10.

In another embodiment, an epimerase that has an amino acid sequence that is homologous to an amino acid sequence encoding a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase includes any epimerase that has an amino acid motif: Gly-Xaa-Xaa-Gly-Xaa-Xaa-Gly, which is found, for example in positions 8 through 14 of the consensus sequence SEQ ID NO:11, in positions 12 through 18 of SEQ ID NO:2, in positions 10 through 16 of SEQ ID NO:4, in positions 14 through 20 of SEQ ID NO:6, in positions

7 through 13 of SEQ ID NO:8, and in positions 9 through 15 of SEQ ID NO:10. Such a motif can be identified by its alignment with the same motif in the above-identified amino acid sequences using a CLUSTAL alignment program. Preferably, such motif is located within the first 25 N-terminal amino acids of the amino acid sequence of the epimerase.

In yet another embodiment, an epimerase encompassed by the present invention includes an epimerase that has a substrate binding site which includes amino acid residues that align using a CLUSTAL alignment program with at least 50% of amino acid positions Asn177, Ser178, Arg187, Arg209, Lys283, Asn165, Ser107, Ser108, Cys109, Asn133, Tyr136 and His179 of SEQ ID NO:4. Alignment with positions Ser107, Tyr136, Asn165, Arg209, is preferably with 100% identity (i.e., exact match of residue, under parameters for alignment).

In another embodiment of the present invention, an epimerase encompassed by the present invention comprises at least 4 contiguous amino acid residues having 100% identity with at least 4 contiguous amino acid residues of an amino acid sequence selected from the group of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8 or SEQ ID NO:10, as determined using a Lipman-Pearson method with Lipman-Pearson standard default parameters or by comparing an alignment using a CLUSTAL program with CLUSTAL standard default parameters. According to the present invention, the term "contiguous" means to be connected in an unbroken sequence. For a first sequence to have "100% identity" with a second sequence means that the first sequence exactly matches the second sequence with no gaps between nucleotides or amino acids.

In another embodiment of the present invention, an epimerase encompassed by the present invention is encoded by a nucleic acid sequence that comprises at least 12 contiguous nucleic acid residues having 100% identity with at least 12 contiguous nucleic acid residues of a nucleic acid sequence selected from the group of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7 or SEQ ID NO:10, as determined using a Lipman-Pearson method with Lipman-Pearson standard default parameters or by comparing an alignment using a CLUSTAL program with CLUSTAL standard default parameters.

In another embodiment of the present invention, an epimerase encompassed by the present invention is encoded by a nucleic acid sequence that hybridizes under stringent

hybridization conditions to a nucleic acid sequence selected from the group of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7 or SEQ ID NO:9. As used herein, stringent hybridization conditions refer to standard hybridization conditions under which nucleic acid molecules are used to identify similar nucleic acid molecules. Such standard conditions are disclosed, for example, in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Labs Press, 1989. Sambrook et al., *ibid.*, is incorporated by reference herein in its entirety (see specifically, pages 9.31-9.62). In addition, formulae to calculate the appropriate hybridization and wash conditions to achieve hybridization permitting varying degrees of mismatch of nucleotides are disclosed, for example, in Meinkoth et al., 1984, *Anal. Biochem.* 138, 267-284; Meinkoth et al., *ibid.*, is incorporated by reference herein in its entirety.

More particularly, stringent hybridization and washing conditions, as referred to herein, refer to conditions which permit isolation of nucleic acid molecules having at least about 70% nucleic acid sequence identity with the nucleic acid molecule being used to probe in the hybridization reaction, more particularly at least about 75%, and most particularly at least about 80%. Such conditions will vary, depending on whether DNA:RNA or DNA:DNA hybrids are being formed. Calculated melting temperatures for DNA:DNA hybrids are 10°C less than for DNA:RNA hybrids. In particular embodiments, stringent hybridization conditions for DNA:DNA hybrids include hybridization at an ionic strength of 6X SSC (0.9 M Na^+) at a temperature of between about 20°C and about 35°C, more preferably, between about 28°C and about 40°C, and even more preferably, between about 35°C and about 45°C. In particular embodiments, stringent hybridization conditions for DNA:RNA hybrids include hybridization at an ionic strength of 6X SSC (0.9 M Na^+) at a temperature of between about 30°C and about 45°C, more preferably, between about 38°C and about 50°C, and even more preferably, between about 45°C and about 55°C. These values are based on calculations of a melting temperature for molecules larger than about 100 nucleotides, 0% formamide and a G + C content of about 40%. Alternatively, T_m can be calculated empirically as set forth in Sambrook et al., *supra*, pages 9.31 to 9.62.

In another embodiment of the present invention, an epimerase encompassed by the present invention is encoded by a nucleic acid sequence that comprises a nucleic acid

sequence selected from the group of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7 or SEQ ID NO:9 or a fragment thereof, wherein the fragment encodes a protein that is capable of catalyzing the conversion of GDP-D-mannose to GDP-L-galactose, such as under physiological conditions. In another embodiment, an epimerase encompassed by the present invention comprises an amino acid sequence selected from the group of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10 or a fragment thereof, wherein the fragment is capable of catalyzing the conversion of GDP-D-mannose to GDP-L-galactose. It is to be understood that the nucleic acid sequence encoding the amino acid sequences identified herein can vary due to degeneracies. As used herein, nucleotide degeneracies refers to the phenomenon that one amino acid can be encoded by different nucleotide codons.

One embodiment of the present invention relates to a method to identify an epimerase that catalyzes conversion of GDP-D-mannose to GDP-L-galactose. Preferably, such a method is useful for identifying the GDP-D-mannose:GDP-L-galactose epimerase which catalyzes the conversion of GDP-D-mannose to GDP-L-galactose in the endogenous (i.e., naturally occurring L-ascorbic acid biosynthetic pathway of microorganisms and/or plants). Such a method can include the steps of: (a) contacting a source of nucleic acid molecules with an oligonucleotide at least about 12 nucleotides in length under stringent hybridization conditions, wherein the oligonucleotide is identified by its ability to hybridize under stringent hybridization conditions to a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3 and SEQ ID NO:5; and, (b) identifying nucleic acid molecules from the source of nucleic acid molecules which hybridize under stringent hybridization conditions to the oligonucleotide. Nucleic acid molecules identified by this method can then be isolated from the source using standard molecular biology techniques. Preferably, the source of nucleic acid molecules is obtained from a microorganism or plant that has an ascorbic acid production pathway. Such a source of nucleic acid molecules can be any source of nucleic acid molecules which can be isolated from an organism and/or which can be screened by hybridization with an oligonucleotide such as a probe or a PCR primer. Such sources include genomic and cDNA libraries and isolated RNA.

In order to screen cDNA libraries from different organisms and to isolate nucleic acid molecules encoding enzymes such as the GDP-D-mannose:GDP-L-galactose epimerase and related epimerases, one can use any of a variety of standard molecular and biochemical techniques. For example, oligonucleotide primers, preferably degenerate primers, can be designed using the most conserved regions of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase nucleic acid sequence, and such primers can be used in a polymerase chain reaction (PCR) protocol to amplify the same or related epimerases, including the GDP-D-mannose:GDP-L-galactose epimerase from the ascorbic acid pathway, from nucleic acids (e.g., genomic or cDNA libraries) isolated from a desired organism (e.g., a microorganism or plant having an L-ascorbic acid pathway). Similarly, oligonucleotide probes can be designed using the most conserved regions of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase nucleic acid sequence and such probe can be used to identify and isolate nucleic acid molecules, such as from a genomic or cDNA library, that hybridize under conditions of low, moderate, or high stringency with the probe.

Alternatively, the GDP-D-mannose:GDP-L-galactose epimerase can be purified from an organism such as *Prototheca*, the N-terminal amino acid sequence can be determined (including the sequence of internal peptide fragments), and this information can be used to design degenerate primers for amplifying a gene fragment from the organism cDNA. This fragment would then be used to probe the cDNA library, and subsequently fragments that hybridize to the probe would be cloned in that organism or another suitable production organism. There is ample precedent for plant enzymes being expressed in an active form in bacteria, such as *E. coli*. Alternatively, yeast are also a suitable candidate for developing a heterologous system for L-ascorbic acid production.

As discussed above in general for increasing the action of an enzyme in the L-ascorbic acid pathway according to the present invention, in one embodiment of the present invention, the action of an epimerase that catalyzes the conversion of GDP-D-mannose to GDP-L-galactose is increased by amplification of the expression (i.e., overexpression) of such an epimerase. Overexpression of an epimerase can be accomplished, for example, by introduction of a recombinant nucleic acid molecule encoding the epimerase. It is preferred that the gene encoding an epimerase according to

the present invention be cloned under control of an artificial promoter. The promoter can be any suitable promoter that will provide a level of epimerase expression required to maintain a sufficient level of L-ascorbic acid in the production organism. Preferred promoters are constitutive (rather than inducible) promoters, since the need for addition of expensive inducers is therefore obviated. The gene dosage (copy number) of a recombinant nucleic acid molecule according to the present invention can be varied according to the requirements for maximum product formation. In one embodiment, the recombinant nucleic acid molecule encoding an epimerase according to the present invention is integrated into the chromosome of the microorganism.

It is another embodiment of the present invention to provide a microorganism having one or more epimerases according to the present invention with improved affinity for its substrate. An epimerase with improved affinity for its substrate can be produced by any suitable method of genetic modification or protein engineering. For example, computer-based protein engineering can be used to design an epimerase protein with greater stability and better affinity for its substrate. See for example, Maulik et al., 1997, *Molecular Biotechnology: Therapeutic Applications and Strategies*, Wiley-Liss, Inc., which is incorporated herein by reference in its entirety.

As noted above, in the method for production of L-ascorbic acid of the present invention, a microorganism having a genetically modified L-ascorbic acid production pathway is cultured in a fermentation medium for production of L-ascorbic acid. An appropriate, or effective, fermentation medium refers to any medium in which a genetically modified microorganism of the present invention, when cultured, is capable of producing L-ascorbic acid. Such a medium is typically an aqueous medium comprising assimilable carbon, nitrogen and phosphate sources. Such a medium can also include appropriate salts, minerals, metals and other nutrients. One advantage of genetically modifying a microorganism as described herein is that although such genetic modifications can significantly alter the production of L-ascorbic acid, they can be designed such that they do not create any nutritional requirements for the production organism. Thus, a minimal-salts medium containing glucose as the sole carbon source can be used as the fermentation medium. The use of a minimal-salts-glucose medium for the L-ascorbic acid fermentation will also facilitate recovery and purification of the L-ascorbic acid product.

In one mode of operation of the present invention, the carbon source concentration, such as the glucose concentration, of the fermentation medium is monitored during fermentation. Glucose concentration of the fermentation medium can be monitored using known techniques, such as, for example, use of the glucose oxidase enzyme test or high pressure liquid chromatography, which can be used to monitor glucose concentration in the supernatant, e.g., a cell-free component of the fermentation medium. As stated previously, the carbon source concentration should be kept below the level at which cell growth inhibition occurs. Although such concentration may vary from organism to organism, for glucose as a carbon source, cell growth inhibition occurs at glucose concentrations greater than at about 60 g/L, and can be determined readily by trial. Accordingly, when glucose is used as a carbon source the glucose concentration in the fermentation medium is maintained in the range of from about 1 g/L to about 100 g/L, more preferably in the range of from about 2 g/L to about 50 g/L, and yet more preferably in the range of from about 5 g/L to about 20 g/L. Although the carbon source concentration can be maintained within desired levels by addition of, for example, a substantially pure glucose solution, it is preferred to maintain the carbon source concentration of the fermentation medium by addition of aliquots of the original fermentation medium. The use of aliquots of the original fermentation medium are desirable because the concentrations of other nutrients in the medium (e.g. the nitrogen and phosphate sources) can be maintained simultaneously. Likewise, the trace metals concentrations can be maintained in the fermentation medium by addition of aliquots of the trace metals solution.

In an embodiment of the fermentation process of the present invention, a fermentation medium is prepared as described above. This fermentation medium is inoculated with
an actively growing culture of genetically modified microorganisms of the present invention in an amount sufficient to produce, after a reasonable growth period, a high cell density. Typical inoculation cell densities are within the range of from about 0.1 g/L to about 15 g/L, preferably from about 0.5 g/L to about 10 g/L and more preferably from about 1 g/L to about 5 g/L, based on the dry weight of the cells. The cells are then grown to a cell density in the range of from about 10 g/L to about 100 g/L preferably from about

20 g/L to about 80 g/L, and more preferably from about 50 g/L to about 70 g/L. The residence times for the microorganisms to reach the desired cell densities during fermentation are typically less than about 200 hours, preferably less than about 120 hours, and more preferably less than about 96 hours.

The microorganisms useful in the method of the present invention can be cultured in conventional fermentation modes, which include, but are not limited to, batch, fed-batch, and continuous. It is preferred, however, that the fermentation be carried out in fed-batch mode. In such a case, during fermentation some of the components of the medium are depleted. It is possible to initiate fermentation with relatively high concentrations of such components so that growth is supported for a period of time before additions are required. The preferred ranges of these components are maintained throughout the fermentation by making additions as levels are depleted by fermentation. Levels of components in the fermentation medium can be monitored by, for example, sampling the fermentation medium periodically and assaying for concentrations. Alternatively, once a standard fermentation procedure is developed, additions can be made at timed intervals corresponding to known levels at particular times throughout the fermentation. As will be recognized by those in the art, the rate of consumption of nutrient increases during fermentation as the cell density of the medium increases. Moreover, to avoid introduction of foreign microorganisms into the fermentation medium, addition is performed using aseptic addition methods, as are known in the art. In addition, a small amount of anti-foaming agent may be added during the fermentation.

The present inventors have determined that high levels of magnesium in the fermentation medium inhibits the production of L-ascorbic acid due to repression of enzymes early in the production pathway, although enzymes late in the pathway (i.e., from L-galactose to L-ascorbic acid) are not negatively affected (See Examples). Therefore, in a preferred embodiment of the method of the present invention, the step of culturing is carried out in a fermentation medium that is magnesium (Mg^{2+}) limited. Even more preferably, the fermentation is magnesium limited during the cell growth phase. Preferably, the fermentation medium comprises less than about 0.5 g/L of Mg^{2+} during the cell growth phase of fermentation, and even more preferably, less than about 0.2 g/L of Mg^{2+}, and even more preferably, less than about 0.1 g/L of Mg^{2+}.

The temperature of the fermentation medium can be any temperature suitable for growth and ascorbic acid production, and may be modified according to the growth requirements of the production microorganism used. For example, prior to inoculation of the fermentation medium with an inoculum, the fermentation medium can be brought to and maintained at a temperature in the range of from about 20°C to about 45°C, preferably to a temperature in the range of from about 25°C to about 40°C, and more preferably in the range of from about 30°C to about 38°C.

It is a further embodiment of the present invention to supplement and/or control other components and parameters of the fermentation medium, as necessary to maintain and/or enhance the production of L-ascorbic acid by a production organism. For example, in one embodiment, the pH of the fermentation medium is monitored for fluctuations in pH. In the fermentation method of the present invention, the pH is preferably maintained at a pH of from about pH 6.0 to about pH 8.0, and more preferably, at about pH 7.0. In the method of the present invention, if the starting pH of the fermentation medium is pH 7.0, the pH of the fermentation medium is monitored for significant variations from pH 7.0, and is adjusted accordingly, for example, by the addition of sodium hydroxide. In a preferred embodiment of the present invention, genetically modified microorganisms useful for production of L-ascorbic acid include acid-tolerant microorganisms. Such microorganisms include, for example, microalgae of the genera *Prototheca* and *Chlorella* (See U.S. Patent No. 5,792,631, *ibid.* and U.S. Patent No. 5,900,370, *ibid.*).

The production of ascorbic acid by culturing acid-tolerant microorganisms provides significant advantages over known ascorbic acid production methods. One such advantage is that such organisms are acidophilic, allowing fermentation to be carried out under low pH conditions, with the fermentation medium pH typically less than about 6. Below this pH, extracellular ascorbic acid produced by the microorganism during fermentation is relatively stable because the rate of oxidation of ascorbic acid in the fermentation medium by oxygen is reduced. Accordingly, high productivity levels can be obtained for producing L-ascorbic acid with acid-tolerant microorganisms according to the methods of the present invention. In addition, control of the dissolved oxygen content to very low levels to avoid oxidation of ascorbic acid is unnecessary. Moreover, this

advantage allows for the use of continuous recovery methods because extracellular medium can be treated to recover the ascorbic acid product.

Thus, the present method can be conducted at low pH when acid-tolerant microorganisms are used as production organisms. The benefit of this process is that at low pH, extracellular ascorbic acid produced by the organism is degraded at a reduced rate than if the fermentation medium was at higher pH. For example, prior to inoculation of the fermentation medium with an inoculum, the pH of the fermentation medium can be adjusted, and further monitored during fermentation. Typically, the pH of the fermentation medium is brought to and maintained below about 6, preferably below 5.5, and more preferably below about 5. The pH of the fermentation medium can be controlled by the addition of ammonia to the fermentation medium. In such cases when ammonia is used to control pH, it also conveniently serves as a nitrogen source in the fermentation medium.

The fermentation medium can also be maintained to have a dissolved oxygen content during the course of fermentation to maintain cell growth and to maintain cell metabolism for L-ascorbic acid formation. The oxygen concentration of the fermentation medium can be monitored using known methods, such as through the use of an oxygen probe electrode. Oxygen can be added to the fermentation medium using methods known in the art, for example, through agitation and aeration of the medium by stirring or shaking. Preferably, the oxygen concentration in the fermentation medium is in the range of from about 20% to about 100% of the saturation value of oxygen in the medium based upon the solubility of oxygen in the fermentation medium at atmospheric pressure and at a temperature in the range of from about 30°C to about 40°C. Periodic drops in the oxygen concentration below this range may occur during fermentation, however, without adversely affecting the fermentation.

The genetically modified microorganisms of the present invention are engineered to produce significant quantities of extracellular L-ascorbic acid. Extracellular L-ascorbic acid can be recovered from the fermentation medium using conventional separation and purification techniques. For example, the fermentation medium can be filtered or centrifuged to remove microorganisms, cell debris and other particulate matter, and L-ascorbic acid can be recovered from the cell free supernate by conventional methods, such

as, for example, ion exchange, chromatography, extraction, solvent extraction, membrane separation, electrodialysis, reverse osmosis, distillation, chemical derivatization and crystallization.

One such example of L-ascorbic acid recovery is provided in U.S. Patent No. 4,595,659 by Cayle, incorporated herein in its entirety be reference, which discloses the isolation of L-ascorbic acid from an aqueous fermentation medium by ion exchange resin adsorption and elution, which is followed by decoloration, evaporation and crystallization. Further, isolation of the structurally similar isoascorbic acid from fermentation medium by a continuous multi-bed extraction system of anion-exchange resins is described by K. Shimizu, *Agr. Biol. Chem.* 31:346-353 (1967), which is incorporated herein in its entirety by reference.

Intracellular L-ascorbic acid produced in accordance with the present invention can also be recovered and used in a variety of applications. For example, cells from the microorganisms can be lysed and the ascorbic acid which is released can be recovered by a variety of known techniques. Alternatively, intracellular ascorbic acid can be recovered by washing the cells to extract the ascorbic acid, such as through diafiltration.

Development of a microorganism with enhanced ability to produce L-ascorbic acid by genetic modification can be accomplished using both classical strain development and molecular genetic techniques, and particularly, recombinant technology (genetic engineering). In general, the strategy for creating a microorganism with enhanced L-ascorbic acid production is to (1) inactivate or delete at least one, and preferably more than one of the competing or inhibitory pathways in which production of L-ascorbic acid is negatively affected (e.g., inhibited), and more significantly to (2) amplify the L-ascorbic acid production pathway by increasing the action of a gene(s) encoding an enzyme(s) involved in the pathway.

In one embodiment, the strategy for creating a microorganism with enhanced L-ascorbic acid production is to amplify the L-ascorbic acid production pathway by increasing the action of GDP-D-mannose:GDP-L-galactose epimerase, as discussed above. Such strategy includes genetically modifying the endogenous GDP-D-mannose:GDP-L-galactose epimerase such that L-ascorbic acid production is increased, and/or expressing/overexpressing a recombinant epimerase that catalyzes the conversion

of GDP-D-mannose to GDP-L-galactose, which includes expression of recombinant GDP-D-mannose:GDP-L-galactose epimerase and/or homologues thereof, and of other recombinant epimerases such as GDP-4-keto-6-deoxy-D-mannose epimerase reductase and epimerases that share structural homology with such epimerase as discussed in detail above.

It is to be understood that a production organism can be genetically modified by recombinant technology in which a nucleic acid molecule encoding a protein involved in the L-ascorbic acid production pathway disclosed herein is transformed into a suitable host which is a different member of the plant kingdom from which the nucleic acid molecule was derived. For example, it is an embodiment of the present invention that a recombinant nucleic acid molecule encoding a GDP-D-mannose:GDP-L-galactose epimerase from a higher plant can be transformed into a microalgal host in order to overexpress the epimerase and enhance production of L-ascorbic acid in the microalgal production organism.

As previously discussed herein, in one embodiment, a genetically modified microorganism can be a microorganism in which nucleic acid molecules have been deleted, inserted or modified, such as by insertion, deletion, substitution, and/or inversion of nucleotides, in such a manner that such modifications provide the desired effect within the microorganism. A genetically modified microorganism is preferably modified by recombinant technology, such as by introduction of an isolated nucleic acid molecule into a microorganism. For example, a genetically modified microorganism can be transfected with a recombinant nucleic acid molecule encoding a protein of interest, such as a protein for which increased expression is desired. The transfected nucleic acid molecule can remain extrachromosomal or can integrate into one or more sites within a chromosome of the transfected (i.e., recombinant) host cell in such a manner that its ability to be expressed is retained. Preferably, once a host cell of the present invention is transfected with a nucleic acid molecule, th nucleic acid molecule is integrated into the host cell genome. A significant advantage of integration is that the nucleic acid molecule is stably maintained in the cell. In a preferred embodiment, the integrated nucleic acid molecule is operatively linked to a transcription control sequence (described below) which can be

A nucleic acid molecule can be integrated into the genome of the host cell either by random or targeted integration. Such methods of integration are known in the art. For example, an *E. coli* strain ATCC 47002 contains mutations that confer upon it an inability to maintain plasmids which contain a ColE1 origin of replication. When such plasmids are transferred to this strain, selection for genetic markers contained on the plasmid results in integration of the plasmid into the chromosome. This strain can be transformed, for example, with plasmids containing the gene of interest and a selectable marker flanked by the 5'- and 3'-termini of the *E. coli lacZ* gene. The *lacZ* sequences target the incoming DNA to the *lacZ* gene contained in the chromosome. Integration at the *lacZ* locus replaces the intact *lacZ* gene, which encodes the enzyme β-galactosidase, with a partial *lacZ* gene interrupted by the gene of interest. Successful integrants can be selected for β-galactosidase negativity.

A genetically modified microorganism can also be produced by introducing nucleic acid molecules into a recipient cell genome by a method such as by using a transducing bacteriophage. The use of recombinant technology and transducing bacteriophage technology to produce several different genetically modified microorganism of the present invention is known in the art.

According to the present invention, a gene, for example the GDP-D-mannose:GDP-L-galactose epimerase gene, includes all nucleic acid sequences related to a natural epimerase gene such as regulatory regions that control production of the epimerase protein encoded by that gene (such as, but not limited to, transcription, translation or post-translation control regions) as well as the coding region itself. In another embodiment, a gene, for example the GDP-D-mannose:GDP-L-galactose epimerase gene, can be an allelic variant that includes a similar but not identical sequence to the nucleic acid sequence encoding a given GDP-D-mannose:GDP-L-galactose epimerase gene. An allelic variant of a GDP-D-mannose:GDP-L-galactose epimerase gene which has a given nucleic acid sequence is a gene that occurs at essentially the same locus (or loci) in the genome as the gene having the given nucleic acid sequence, but which, due to natural variations caused by, for example, mutation or recombination, has a similar but not identical sequence. Allelic variants typically encode proteins having similar activity to that of the protein encoded by the gene to which they are being

compared. Allelic variants can also comprise alterations in the 5' or 3' untranslated regions of the gene (e.g., in regulatory control regions). Allelic variants are well known to those skilled in the art and would be expected to be found within a given microorganism or plant and/or among a group of two or more microorganisms or plants.

In accordance with the present invention, an isolated nucleic acid molecule is a nucleic acid molecule that has been removed from its natural milieu (i.e., that has been subject to human manipulation). As such, "isolated" does not reflect the extent to which the nucleic acid molecule has been purified. An isolated nucleic acid molecule can include DNA, RNA, or derivatives of either DNA or RNA. There is no limit, other than a practical limit, on the maximal size of a nucleic acid molecule in that the nucleic acid molecule can include a portion of a gene, an entire gene, or multiple genes, or portions thereof.

An isolated nucleic acid molecule of the present invention can be obtained from its natural source either as an entire (i.e., complete) gene or a portion thereof capable of forming a stable hybrid with that gene. An isolated nucleic acid molecule can also be produced using recombinant DNA technology (e.g., polymerase chain reaction (PCR) amplification, cloning) or chemical synthesis. Isolated nucleic acid molecules include natural nucleic acid molecules and homologues thereof, including, but not limited to, natural allelic variants and modified nucleic acid molecules in which nucleotides have been inserted, deleted, substituted, and/or inverted in such a manner that such modifications provide the desired effect within the microorganism. A structural homologue of a nucleic acid sequence has been described in detail above. Preferably, a homologue of a nucleic acid sequence encodes a protein which has an amino acid sequence that is sufficiently similar to the natural protein amino acid sequence that a nucleic acid sequence encoding the homologue is capable of hybridizing under stringent conditions to (i.e., with) a nucleic acid molecule encoding the natural protein (i.e., to the complement of the nucleic acid strand encoding the natural protein amino acid sequence). A nucleic acid molecule homologue encodes a protein homologue. As used herein, a homologue protein includes proteins in which amino acids have been deleted (e.g., a truncated version of the protein, such as a peptide), inserted, inverted, substituted and/or derivatized (e.g., by glycosylation, phosphorylation, acetylation, myristoylation, prenylation, palmitation,

amidation and/or addition of glycosylphosphatidyl inositol) in such a manner that such modifications provide the desired effect on the protein and/or within the microorganism (e.g., increased or decreased action of the protein).

A nucleic acid molecule homologue can be produced using a number of methods known to those skilled in the art (see, for example, Sambrook et al., *ibid.*). For example, nucleic acid molecules can be modified using a variety of techniques including, but not limited to, classic mutagenesis techniques and recombinant DNA techniques, such as site-directed mutagenesis, chemical treatment of a nucleic acid molecule to induce mutations, restriction enzyme cleavage of a nucleic acid fragment, ligation of nucleic acid fragments, PCR amplification and/or mutagenesis of selected regions of a nucleic acid sequence, synthesis of oligonucleotide mixtures and ligation of mixture groups to "build" a mixture of nucleic acid molecules and combinations thereof. Nucleic acid molecule homologues can be selected from a mixture of modified nucleic acids by screening for the function of the protein encoded by the nucleic acid and/or by hybridization with a wild-type gene.

Although the phrase "nucleic acid molecule" primarily refers to the physical nucleic acid molecule and the phrase "nucleic acid sequence" primarily refers to the sequence of nucleotides on the nucleic acid molecule, the two phrases can be used interchangeably, especially with respect to a nucleic acid molecule, or a nucleic acid sequence, being capable of encoding a gene involved in an L-ascorbic acid production pathway.

Knowing the nucleic acid sequences of certain nucleic acid molecules of the present invention allows one skilled in the art to, for example, (a) make copies of those nucleic acid molecules and/or (b) obtain nucleic acid molecules including at least a portion of such nucleic acid molecules (e.g., nucleic acid molecules including full-length genes, full-length coding regions, regulatory control sequences, truncated coding regions). Such nucleic acid molecules can be obtained in a variety of ways including traditional cloning techniques using oligonucleotide probes to screen appropriate libraries or DNA and PCR amplification of appropriate libraries or DNA using oligonucleotide primers. Preferred libraries to screen or from which to amplify nucleic acid molecule include bacterial and yeast genomic DNA libraries, and in particular, microalgal genomic DNA libraries. Techniques to clone and amplify genes are disclosed, for example, in Sambrook et al., *ibid.*

The present invention includes a recombinant vector, which includes at least one isolated nucleic acid molecule of the present invention, inserted into any vector capable of delivering the nucleic acid molecule into a host microorganism of the present invention. Such a vector can contain nucleic acid sequences that are not naturally found adjacent to the isolated nucleic acid molecules to be inserted into the vector. The vector can be either RNA or DNA and typically is a plasmid. Recombinant vectors can be used in the cloning, sequencing, and/or otherwise manipulating of nucleic acid molecules. One type of recombinant vector, referred to herein as a recombinant molecule and described in more detail below, can be used in the expression of nucleic acid molecules. Preferred recombinant vectors are capable of replicating in a transformed bacterial cells, yeast cells, and in particular, in microalgal cells.

Transformation of a nucleic acid molecule into a cell can be accomplished by any method by which a nucleic acid molecule can be inserted into the cell. Transformation techniques include, but are not limited to, transfection, electroporation, microinjection and biolistics.

A recombinant cell is preferably produced by transforming a host cell with one or more recombinant molecules, each comprising one or more nucleic acid molecules operatively linked to an expression vector containing one or more transcription control sequences. The phrase, operatively linked, refers to insertion of a nucleic acid molecule into an expression vector in a manner such that the molecule is able to be expressed when transformed into a host cell. As used herein, an expression vector is a DNA or RNA vector that is capable of transforming a host cell and of effecting expression of a specified nucleic acid molecule. Preferably, the expression vector is also capable of replicating within the host cell. In the present invention, expression vectors are typically plasmids. Expression vectors of the present invention include any vectors that function (i.e., direct gene expression) in a yeast host cell, a bacterial host cell, and preferably a microalgal host cell.

Nucleic acid molecules of the present invention can be operatively linked to expression vectors containing regulatory sequences such as transcription control sequences, translation control sequences, origins of replication, and other regulatory sequences that are compatible with the recombinant cell and that control the expression

of nucleic acid molecules of the present invention. In particular, recombinant molecules of the present invention include transcription control sequences. Transcription control sequences are sequences which control the initiation, elongation, and termination of transcription. Particularly important transcription control sequences are those which control transcription initiation, such as promoter, enhancer, operator and repressor sequences. Suitable transcription control sequences include any transcription control sequence that can function in yeast or bacterial cells or preferably, in microalgal cells. A variety of such transcription control sequences are known to those skilled in the art.

It may be appreciated by one skilled in the art that use of recombinant DNA technologies can improve expression of transformed nucleic acid molecules by manipulating, for example, the number of copies of the nucleic acid molecules within a host cell, the efficiency with which those nucleic acid molecules are transcribed, the efficiency with which the resultant transcripts are translated, and the efficiency of post-translational modifications. Recombinant techniques useful for increasing the expression of nucleic acid molecules of the present invention include, but are not limited to, operatively linking nucleic acid molecules to high-copy number plasmids, integration of the nucleic acid molecules into the host cell chromosome, addition of vector stability sequences to plasmids, substitutions or modifications of transcription control signals (e.g., promoters, operators, enhancers), substitutions or modifications of translational control signals, modification of nucleic acid molecules of the present invention to correspond to the codon usage of the host cell, deletion of sequences that destabilize transcripts, and use of control signals that temporally separate recombinant cell growth from recombinant enzyme production during fermentation. The activity of an expressed recombinant protein of the present invention may be improved by fragmenting, modifying, or derivatizing nucleic acid molecules encoding such a protein.

The following experimental results are provided for the purposes of illustration and are not intended to limit the scope of the invention.

EXAMPLES

Example 1

The present example describes the elucidation of the pathway from glucose to L-ascorbic acid through GDP-D-mannose in plants.

Since the present inventors have previously shown that *Prototheca* makes L-ascorbic acid (AA) from glucose, it was worthwhile to examine cultures for some of the early conversion products of glucose. In the past, the present inventors had concentrated on pathways from glucose to organic acids, based on the published pathway of L-ascorbic acid synthesis in animals and proposed pathways in plants. The present inventors demonstrate herein that the pathway from glucose to L-ascorbic acid involves not organic acids, but rather sugar phosphates and nucleotide diphosphate sugars (NDP-sugars).

Prior to the present invention, it was known that all cells synthesize polysaccharides by first forming NDP-sugars. The sugar moiety is then incorporated into polymer, while the cleaved NDP is recycled. A variety of polysaccharides are known, and are usually named based on the relative proportions of the sugar residues in the polymers. For example, a "galactomannan" contains mostly galactose, and to a lesser degree, mannose residues. The "biopolymer" from *Prototheca* strains isolated by the present inventors was analyzed and found to be 80% D-galactose, 18% rhamnose (D- or L-configuration not determined), and 2% L-arabinose. The present inventors provide evidence herein of how the respective NDP-sugars that make up the *Prototheca* biopolymer are formed, and what correlations exist between L-ascorbic acid synthesis and the formation of the NDP-sugar forms of the sugar residues found in the biopolymer.

The common NDP-sugar UDP-glucose is shown in Fig. 2B. This is formed in plants from glucose-l-P by the action of UDP-D-glucose pyrophosphorylase. UDP-glucose can be epimerized in plants to form UDP-D-galactose, using UDP-D-glucose-4-epimerase. UDP-D-galactose can also be formed by phosphorylation of D-galactose by galactokinase to form D-galactose-l-P, which can be converted to UDP-D-galactose by UDP-D-galactose pyrophosphorylase. These known routes were believed to account for the D-galactose in the *Prototheca* biopolymer. The UDP-L-arabinose can be formed by known reactions beginning with the oxidation of UDP-D-glucose to UDP-D-glucuronic acid (by UDP-D-glucose dehydrogenase), decarboxylation to UDP-D-xylose, and epimerization to UDP-L-arabinose. This accounts for th arabinose residues in the

biopolymer. UDP-L-rhamnose is known to be formed from UDP-D-glucose, thus all three of the sugar moieties in the *Prototheca* biopolymer can be accounted for by a pathway through glucose-1-P and UDP-glucose. Alternatively, if the rhamnose in the biopolymer is D-rhamnose, it is not formed via UDP-D-glucose, but by oxidation of GDP-D-mannose (See Fig. 1).

GDP-D-rhamnose is formed by converting glucose, in turn, to D-glucose-6-P, D-fructose-6-P, D-mannose-6-P, D-mannose-1-P, GDP-D-mannose, and GDP-D-rhamnose. It was of interest to the present inventors that this route passes through GDP-D-mannose. Exogenous mannose is known to be converted to D-mannose-6-P in plants, and can enter the path above. D-mannose is converted to L-ascorbic acid by *Prototheca* cells cultured by the present inventors as well or better than glucose (see Example 4). The mechanism of conversion, in *Chlorella pyrenoidosa* , of GDP-D-mannose to GDP-L-galactose by GDP-D-mannose:GDP-L-galactose epimerase, has been known for years (See, Barber, 1971, *Arch. Biochem. Biophys.* 147:619-623, incorporated herein by reference in its entirety). The present inventors have discovered herein that L-galactose and L-galactono-γ-lactone are rapidly converted to L-ascorbic acid by strains *of Prototheca* and *Chlorella pyrenoidosa*. Prior to the present invention, it was known that L-galactono-γ-lactone is converted to L-ascorbic acid in several plant systems, but the synthesis steps prior to this step were unknown. Based on the published literature and the present experimental evidence, the present inventors have determined that the L-ascorbic acid biosynthetic pathway in plants passes through GDP-D-mannose and involves sugar phosphates and NDP-sugars. The proposed pathway is shown in Fig. 1. Salient points relevant to the design and production of genetically modified microorganisms useful in the present method include:

1. The enzymes leading from D-glucose to D-fructose-6-P are well known enzymes in the first, uncommitted steps of glycolysis.

2. The enzymes involved in the conversion of D-fructose-6-P to GDP-D-mannose have been well characterized in plants, yeast, and bacteria, particularly *Azotobacter vinelandii and Pseudomonas aeruginosa,* which convert GDP-D-mannose to GDP-D-mannuronic acid, which is the precursor for alginate (See for example, Sa-Correia et al., 1987, *J. Bacteriol.* 169:3224-3231; Koplin et al., 1992, *J. Bacteriol.* 174:191-199; Oesterhelt et al., 1996, *Plant Science* 121:19-27; Feingold et al., 1980, The

Biochemistry of Plants: Vol 3: Carbohydrates, structure and function, P.K. Stampf & E.E. Conn, eds., Academic Press, New York, pp. 101-170; Smith et al., 1992, *Mol. Cell Biol.* 12:2924-2930; Boles et al., 1994, *Eur. J. Med.* 220:83-96; Hashimoto et al., 1997, *J. Biol. Chem.* 272:16308-16314, all of which are incorporated herein by reference in their entirety).

3. Barber (1971, *supra*, and 1975) identified in *Chlorella pyrenoidosa* the enzyme activities for the conversion of GDP-D-mannose to GDP-L-galactose and L-galactose-l-P.

4. The present inventors have shown herein the rapid conversion of L-galactose and L-galactono-γ-lactone to L-ascorbic acid by *Prototheca* cells.

5. L-galactono-γ-lactone and L-galactonic acid can be interconverted in solution by changing the pH of the solution; addition of base shifts the equilibrium to L-galactonic acid, while addition of acid shifts the equilibrium to the lactone. Cells may have an enzymatic means for this conversion in addition to this non-enzymatic route.

6. In plants, GDP-L-fucose is also formed from GDP-D-mannose, presumably for incorporation into polysaccharide. Roberts (1971) fed labeled D-mannose to corn root tips and found the label in polysaccharide, specifically in the residues of D-mannose, L-galactose, and L-fucose. No label was detected in D-glucose, D-galactose, L-arabinose, or D-xylose. *Prototheca and C. pyrenoidosa* cells have the ability to convert L-fucose (6-deoxy-L-galactose) to a dipyridyl-positive product that was shown by HPLC not to be L-ascorbic acid. The present inventors believe that it is was the 6-deoxy analog of L-ascorbic acid.

Example 2

This example shows that in *Prototheca*, like other plants (Loewus, F.A. 1988. In: J. Priess (ed.), The Biochemistry of Plants, 14:85-107. New York, Academic Press) and the green microalga *Chlorella pyrenoidosa* (Renstrom, *et al.*, 1983. Plant Sci. Lett. 28:299-305), ascorbic acid (AA) production from glucose proceeds by a biosynthetic pathway that allows retention of the configuration of the carbon skeleton of glucose.

Cultures of the strain UV77-247 were grown to moderate cell density in shake flasks with 1-^{13}C-labeled glucose as 10% of the total glucose (40 g/L). Incubation was

as per the standard Mg-limited screen (see Example 3). The culture supernates were clarified, deionized to remove salts, lyophilized, and subjected to nuclear magnetic resonance (nmr) analysis to determine where in the AA molecule the ^{13}C was located. In each case, approximately 85% of the label was found at the C-1 position of AA, with most of the remaining label at the C-6 position. This strongly indicated that AA is synthesized from glucose by a pathway that retains the carbon chain configuration, *i. e.*, C-1 of glucose becomes C-1 of AA. This has typically been observed in plants (Loewus, F.A. 1988. Ascorbic acid and its metabolic products. In: The Biochemistry of Plants, ed. J. Priess, 14:85-107. New York, Academic Press). Animals (Mapson, L.W. and F.A. Isherwood 1956. Biochem. J. 64:151-157; Loewus, F.A. 1960. J. Biol. Chem. 235(4):937-939) and protists such as *Euglena* (Shigeoka, S., *et al.*, 1979. J. Nutr. Sci. Vitaminol. 25:299-307), on the other hand, synthesize AA by a pathway that involves the inversion of configuration, *i. e.*, C-1 of glucose becomes C-6 of AA. Demonstration of the inversion/non-inversion nature of the pathway was an important step in determining the pathway of AA biosynthesis since the two types of pathways require different types of enzymatic reactions. The label found at C-6 of AA is thought to be due to metabolism of glucose and subsequent gluconeogenesis. The metabolism of glucose in glycolysis proceeds through triose-phosphate intermediates. After this, the C-1 and C-6 carbons of glucose become biochemically equivalent. Hexose phosphates can be regenerated from the triose phosphates by gluconeogenesis, which is essentially a reversal of the degradative pathway. Consequently, metabolism of C-1-labeled glucose to triose phosphates with subsequent gluconeogenesis would result in the formation of hexose phosphate molecules labeled at either or both C-1 and C-6. If those hexose phosphates were precursors to AA, one would expect the AA to be similarly labeled. Consistent with this type of "isotopic mixing" is the observation that sucrose obtained from 1-^{13}C-labeled glucose was labeled at positions 1, 6, 1' and 6'.

Glucose can also be metabolized by the pentose phosphate pathway, the overall balanced equation for which is:

$$\text{3 Glucose-6-phosphate} \rightarrow \text{2 Fructose-6-phosphate} + \text{Glyceraldehyde-3-phosphate} + 3\ CO_2$$

Based on the known biochemistry, it would then be expected that the label at each of the carbons in glucose (Table 1 left column) would appear at the positions for the other molecules shown, and that these patterns would be reflected in the AA formed from C-2- and C-3-labeled glucose.

TABLE 1

Predicted Carbon Labeling of Metabolites of Glucose in the Pentose Phosphate Pathway

Labeled Glucose Carbon	Position of Labeled Carbon in:			
	CO_2	F6P(1)	F6P(2)	G3P
1	+	-	-	-
2	-	1,3	1	-
3	-	2	2,3	-
4	-	4	4	1
5	-	5	5	2
6	-	6	6	3

AA recovered from cultures fed glucose labeled at C-2 or C-3 was also analyzed for its labeling patterns (Table 2).

TABLE 2

Labeling Pattern in AA after Cells were Fed 2-^{13}C and 3-^{13}C-glucose

Carbon Position in AA	Isotopic enhancement after growth on:	
	C-2 labeled glucose	C-3 labeled glucose
1	1.0	0.4
2	10.0	0.9
3	0.5	9.9
4	0	2.8
5	2.2	0.2
6	0	0

The data above again suggest a pathway from glucose to AA that proceeds by retention of configuration. As in the experiments with C-1 labeled glucose, approximately one-fifth of the label is present in "mirror image" position to the glucose label (C-5 for C-2 labeled glucose and C-4 for C-3 labeled glucose), indicating levels of gluconeogenesis consistent with those previously observed.

The small, but significant amount of enhancement observed in other positions is consistent with flux through the pentose phosphate pathway. As predicted above, carbon

flux through this pathway would result in isotopic enhancement at positions 1 and 3 when cells were grown on 2-^{13}C glucose and enhancement at position 2 when cells were grown on 3-^{13}C glucose. This is indeed observed. That there is twice as much enhancement at C-1 as there is at C-3 after growth on 2-^{13}C glucose is also predicted. These data indicate a small but measurable amount of carbon flux through the pentose phosphate pathway.

Example 3

This example shows the methods for generating, screening and isolating mutants of *Prototheca* with altered AA productivities compared to the starting strain ATCC 75669.

ATCC No. 75669, identified as *Prototheca moriformis* RSP1385 (unicellular green microalga), was deposited on February 8, 1994, with the American Type Culture Collection (ATCC), Rockville, Maryland, 20852, USA, under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure. Initial screening of *Prototheca* species and strains was reported in U.S. Patent No. 5,900,370, *ibid.* Table 3 lists the formulations of the media for growth and maintenance of the strains. Glucose for fermentors was supplied as glucose monohydrate and calculated on an anhydrous basis. The recipe for the trace metals solution is given in Table 4. The standard growth temperature was 35°C. All organisms were cultured axenically.

TABLE 3
Media for Growth and Maintenance of *Prototheca* Strains
All quantities are in g/L unless otherwise specified

Ingredient	Liquid		Agar		
	Standard	Mg-limiting	Slants	Ferrozine Plates	Standard Plates
Potassium phosphate monobasic	1.3	1.3	2.0	0.27	2.0
Potassium phosphate dibasic	3.8	3.8	2.0	1.4	2.0
Trisodium citrate dihydrate	7.7	7.7	2.6	1.3	2.6
Magnesium sulfate heptahydrate	0.40	0.02	0.4	0.01	0.4
Ammonium sulfate	3.7	3.7	1.0	1.0	1.0
Trace Metals Solution	2 mL	2 mL	2 mL	2 mL	2 mL
Ferrous sulfate heptahydrate	1.5 mg	4.5 mg	1.5 mg	-	1.5 mg
Calcium chl ride dihydrate	-	0.25	-	-	-

Ingredient	Liquid		Agar		
	Standard	Mg-limiting	Slants	Ferrozine Plates	Standard Plates
Manganous sulfate monohydrate	-	0.08	-	-	-
Yeast extract	-	-	2.5	-	-
Agar	-	-	15	15 (Noble)	15
pH before autoclaving	7.2	7.2	7.2	7.2	7.2
Autoclave, then add					
Copper sulfate, pentahydrate, 100 g/L	-	-	-	2 mL	-
40 g/L Ferrozine in 5 mM phosphate (pH 7.5 final)	-	-	-	8.8 mL	-
Ferric ammonium sulfate dodecahydrate, 40 g/L	-	-	-	3.8 mL	-
50% glucose with 25 mg/L thiamine HCl	40 mL	60 mL	10 mL	10 mL	10 mL

TABLE 4

Trace Metals Solution

Compound	Molecular Weight	Conc. of Individ. Solutions, g/L	mL Indiv. Stock per liter of Working Stock
Distilled Water	—	—	823
Hydrochloric Acid	—	Conc.	20
Cobalt Chloride hexahydrate	237.9	24.0	6.5
Boric acid	61.8	38.1	24
Zinc sulfate heptahydrate	287.5	35.3	50
Manganous sulfate monohydrate	169.0	24.6	50
Sodium molybdate dihydrate	242.0	23.8	2.0
Calcium chloride dihydrate	147.0	—	11.4 g
Vanadyl sulfate dihydrate	199.0	10.0	8.0
Nickel nitrate hexahydrate	290.8	5.0	8.0
Sodium selenite	173.0	5.0	8.0

Mutant isolates were generated by treatment with one or more of the following agents: nitrous acid (NA); ethyl methane sulfonate (EMS); or ultraviolet light (UV). Typically, glucose-depleted cells grown in standard liquid medium were washed and resuspended in 25 mM phosphate buffer, pH 7.2, diluted to approximately 10^7 colony-forming units per mL (cfu/mL), exposed to the mutagen to achieve about 99% kill, incubated 4-8 hours in the dark, and spread onto standard agar medium, or agar media containing differential agents.

Some mutant colonies on standard agar medium were picked randomly and subcultured to master plates. Other isolation plates were inverted over chloroform to lyse cells on the surface of the colonies and allow them to release AA. Released AA was detected by spraying the treated plates with a solution of 2,6-dichrorophenol-indophenol (1.25 g/L in 70% EtOH). The ability of AA to reduce this blue redox dye to its colorless form is the basis for a standard assay of AA (Omaye, *et al.*, 1979. Meth. Enzymol. 62:3-11.). Colonies derived from mutagenized cells were saved to master plates for further evaluation if their clear halos were significantly larger than the halos typical of the other mutants in that group. Other mutagenized cells were spread onto plates containing an AA detection system incorporated directly into the agar. This system is based on the ability of AA to reduce ferric iron to ferrous iron. The compound ferrozine (3-(2-pyridyl)-5,6- bis(4-phenylsulfonic acid)-1,2,4-triazine) was present in the agar to complex with the ferrous iron and give a violet color reaction. The ferrozine agar formulation is shown in Table 3. Colonies giving the darkest color reactions were master-plated. When screening for non-AA-producing strains (blocked mutants), white colonies were chosen against a background of relatively dark colonies.

For primary screening of tube cultures, cells were inoculated from master plates into 4 mL of Mg-limiting medium in 16 x 125 mm test tubes, and tubes were shaken in a slanted position on a rotary shaker at 300 rpm for four days. After both three and four days of incubation aliquots were removed for AA assay and cell density determination. Those for AA assay were centrifuged at 1500 x g for 5 min and the resulting supernates were removed for either colorimetric assay or high pressure liquid chromatography (HPLC). Promising isolates were retested in tube culture. Those passing the tube screen were tested in shake flasks.

For secondary screening of flask cultures, cells were inoculated into 50 mL of standard flask medium in 250 mL baffled shake flasks, and incubated on a rotary shaker at 180 rpm until glucose depletion (24-48 hours). A second series of flasks of Mg-sufficient standard medium was inoculated from the first set to a cell density of 0.15 A_{620}, and incubated for 24 hours. A third series of Mg-limiting flask medium was inoculated from the second set by a 1/50 dilution and incubated for 96 hours. Flasks were sampled for AA analysis and cell density measurements during this time as required.

Aliquots for supernatant AA analysis were centrifuged at 5000 x g for 5 min. Aliquots for total whole broth AA analysis were first extracted for 15 min with an equal volume of 5% trichloroacetic acid (TCA) before centrifugation. Aliquots of the resulting supernates were removed for either colorimetric assay or HPLC analysis.

For colorimetric assay of AA, a modification of the method of Omaye, *et al.* (1979. Meth. Enzymol. 62:3-11) was used. Twenty-five µL aliquots of culture supernates were added to wells of 96-well microplates, and 125 µL of color reagent was added. The color reagent consisted of four parts 0.5% aqueous 2,2'-dipyridyl and one part 8.3 mM ferric ammonium sulfate in 27 % (v/v) *o*-phosphoric acid, the two components being mixed immediately before use. After one hour, the absorbance at 520 nm was read. AA concentration was calculated by comparison of the absorbances of AA standards.

HPLC analysis was based on that of Running, *et al.*, (1994). Supernates were chromatographed on a Bio-Rad HPX-87H organic acid column (Bio-Rad Laboratories, Richmond, CA) with 13 mM nitric acid as solvent, at a flow rate of 0.7 mL/min at room temperature. Detection was at either 254 nm using a Waters 441 detector (Millipore Corp., Milford, MA), or at 245 nm using a Waters 481 detector. This system can distinguish between the L- and D- isomers of AA.

For dry weight determinations of cell density, 5 mL whole broth samples were centrifuged at 5000 x g for 5 min, washed once with distilled water, and the pellet was washed into a tared aluminum weighing pan. Cells were dried for 8-24 h at 105°C. Cell weight was calculated by difference.

Table 5 shows the abilities of various mutants of *Prototheca* to synthesize AA.

TABLE 5

AA Synthesizing Ability of Various *Prototheca* Mutants in Flask Screen

Strain	Specific AA Formation, mg AA per L/Culture A_{620}, during Mg-limited Incubation	
	2 Days Incubation	4 Days Incubation
ATCC 75669	22	35
EMS13-4	79	166
UV213-1	0	0
UV218-1	0	0
UV244-1	0	0

Strain	Specific AA Formation, mg AA per L/Culture A_{620}, during Mg-limited Incubation	
	2 Days Incubation	4 Days Incubation
UV244-15	58	68
UV77-247	56	83
UV140-1	67	100
UV164-6	91	131
NA21-14	27	78
UV82-21	0	0
UV127-10	50	95
SP2-3	3	4

The genealogy of these isolates is presented graphically in the "family tree" of Fig. 3. ATCC No. _____, identified as *Prototheca moriformis* EMS13-4 (unicellular green microalga), was deposited on May 25, 1999, with the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA 20110, USA, under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure. ATCC No. _____, identified as *Prototheca moriformis* UV127-10 (unicellular green microalga), was deposited on May 25, 1999, with the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA 20110, USA, under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure. ATCC No. _____, identified as *Prototheca moriformis* SP2-3 (unicellular green microalga), was deposited on May 25, 1999, with the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA 20110, USA, under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure.

Example 4

The following example shows that both growing and resting cells of *Prototheca* can rapidly convert L-galactose and L-galactono-γ-lactone to AA, and that conversion of D-mannose to AA by *Prototheca* is more rapid than conversion of D-glucose.

Shake flask cultures of the mutant strain UV77-247 were grown to glucose depletion in standard liquid medium (Table 3). Cells were washed twice and resuspended

Cell suspensions were incubated for 24 hours at 35° C with shaking, and the entire suspension was extracted with TCA as above and assayed for AA.

Tables 6-8 show that both growing and resting cells of strain UV77-247 can rapidly convert L-galactose and L-galactono-γ-lactone to AA. In these experiments, D-fructose and D-galactose were converted to AA at the same rate as D-glucose, suggesting that they are metabolized to AA through the same route as D-glucose. None of the organic acids suggested in the literature to be intermediates in the biosynthesis of AA were converted to AA, including sorbosone, which has been proposed as an intermediate by Saito *et al.*(1990 Plant Physiol. 94:1496-1500).

TABLE 6

Conversion of Compounds by Resting Cells of Strain UV77-247

Substrate (50 mM)	Total AA, mg/L	AA Relative to No Substrate Control
L-galactose	965	623
L-galactono-γ-lactone	818	476
D-fructose	590	248
D-glucosone	589	247
D-glucose	584	242
D-galactose	542	200
D-glucose (10 mM)	388	46
D-gluconolactone	382	40
D-gulono-γ-lactone	366	24
D-glucuronate	364	22
L-sorbosone	342	0
None	342	0
2-keto-D-gluconic acid	341	-1
D-isoascorbic acid (10 mM)	330	-12
D-glucuronolactone	329	-13
D-gluconic acid	309	-33
D-galacturonic acid	297	-45
L-idonate	296	-46

Since strain UV77-247 converted L-galactose and L-galactono-γ-lactone to AA much more rapidly than it did glucose, it suggests that these compounds are intermediates in the AA biosynthetic pathway and that they are "downstream" from glucose.

The data in Tables 7 and 8 also show that growing and resting cells of UV77-247 consistently convert D-mannose to AA at a rate greater than that of glucose.

TABLE 7

Conversion of Compounds to AA by Resting Cells of Strain UV77-247

Compound	Ascorbic Acid, mg/L		
	25.5 h	30 h	47 h
L-galactose	667	718	620
L-galactono-γ-lactone	644	681	749
D-glucosone	465	462	354
D-mannose	448	462	399
D-fructose	402	408	367
d-glucose	395	404	351
D-galactose	352	361	337
none	287	288	258

TABLE 8

Conversion of Compounds to AA by Growing Cells of Strain UV77-247

Compound	Ascorbic Acid, mg/L		A_{620}	AA/A_{620}
	25.5 h	44 h		
L-galactose	249	506	4.5	112
D-mannose	228	488	5.6	87
L-galactono-γ-lactone	214	342	5.0	68
D-glucose	178	398	5.9	67
D-fructose	181	383	5.9	65
D-glucosone	176	362	5.7	64
D-galactose	185	380	5.9	64
none	182	249	4.4	57
D-gluconic acid (K)	178	262	5.0	52
L-idonate (Na)	182	232	4.7	49
2-keto-D-gluconic acid	182	255	5.3	48
2-deoxy-D-glucose	181	227	4.8	47
D-glucuronic acid lactone	165	218	5.0	44
D-glucuronic acid (Na)	173	241	5.6	43
L-gulono-γ-lactone	152	195	5.0	39
L-sorbosone	178	160	4.7	34
D-glucono-δ-lactone	130	190	5.7	33
D-galacturonic acid	130	180	6.0	30

These cells converted L-galactose, L-galactono-γ-lactone and D-mannose to AA more rapidly than they did glucose, suggesting that mannose exerts its effect in the biosynthetic pathway "downstream" from glucose.

Example 5

Using the methods described above, a collection of mutants was assembled. The specific AA formation for representative mutants are shown in Table 5. The genealogy of these isolates is presented graphically in the "family tree" of Fig. 3.

These isolates were tested for their ability to convert compounds which could be converted to AA by strain UV77-247. Testing was done as in Example 4. Results are shown in Table 9.

TABLE 9

Conversion of Compounds to AA by Resting Cells of Mutant Strains of *Prototheca* of Varying Abilities to Synthesize AA

	Absolute AA, mg/L					
Strain	Buffer	Glucose	L-galactose	L-gal-γ-lact.	Mannose	Fructose
EMS13-4	53	97	191	173	139	ND
UV127-10	45	140	213	140	128	143
SP2-3	19	19	204	146	24	27
NA21-14	61	80	147	158	118	115
UV82-21	15	16	183	175	18	17
UV213-1	16	15	170	135	17	16
UV218-1	16	18	136	176	19	21
UV244-1	16	16	164	162	16	16
UV244-15	26	77	30	21	94	89
UV244-16	28	64	53	53	53	66

ND = Not Determined

These data suggest that the mutational blocks in those strains which convert fructose and mannose to AA poorly are before ("upstream" from) L-galactose and L-galactono-γ-lactone in the pathway.

Example 6

The following example shows that magnesium inhibits early steps in the production of AA.

To address the question of whether magnesium actually inhibits AA synthesis, strain NA45-3 (ATCC 209681) was grown in magnesium (Mg)-limited and Mg-sufficient medium. ATCC No. 209681, identified as *Prototheca moriformis* NA45-3 (Source:

repeated mutagenesis of ATCC No. 75669; Eucaryotic alga. Division Chlorophyta, Class Chlorophyceae, Order Chlorococcales), was deposited on March 13, 1998, with the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA 20110, USA, under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure. Cells from both cultures were harvested and resuspended in the cell-free supernate from the Mg-limited culture, and to half of each cell suspension additional magnesium was added in order to bring the level in the suspension to the Mg-sufficient level. The four conditions under which assays were run were as follows.

TABLE 10

Conditions Used to Test the Effect of Magnesium on AA Production

Condition	Magnesium concentration, g/L, during:	
	Growth	Assay
1Mg>1Mg	0.02	0.02
1Mg>10Mg	0.02	0.2
10Mg>1Mg	0.2	0.02
10Mg>10Mg	0.2	0.2

Substrates previously shown to lead to the formation of AA, namely D-glucose, D-glucosone, D-fructose. D-galactose, D-mannose, and L-galactono-γ-lactone, were added at 20 g/L to the four cell suspensions. Accumulation of AA after 24 hours was measured and compared to a control in which no substrate was added. The results of this study are shown graphically in Fig. 4.

When cells growing under magnesium-limited conditions were incubated with substrates in low-magnesium broth (1Mg>1Mg condition), all showed significant and similar accumulation of AA over the control condition. When the same cells were incubated in high magnesium broth (1Mg>10Mg condition), the accumulation of AA was reduced about 40% for all substrates except D-mannose and L-galactono-γ-lactone, suggesting that 1) the rate-limiting step in the conversion of D-glucose, D-glucosone, D-fructose, and D-galactose to AA is inhibited by magnesium or 2) magnesium stimulates an enzyme which results in the conversion of these compounds to some other compound(s), reducing the amount of substrate available for AA synthesis. On the other

hand, conversion of D-mannose and L-galactono-γ-lactone appeared to be unaffected by the presence of magnesium in the resuspension buffer, indicating that either 1) magnesium-inhibited enzymes are not involved in the conversion of these substrates to AA or 2) D-mannose and L-galactono-γ-lactone enter the pathway far enough downstream from the point where they can be siphoned off by side reactions involving Mg-requiring enzymes.

When cells were grown under magnesium-sufficient conditions, very little AA accumulation from any of the D-sugars was observed, regardless of the level of magnesium in the resuspension broth. Accumulation of AA from L-galactono-γ-lactone, however, was enhanced over that observed when cells are grown in Mg-limited conditions. This suggests that enzymes early in the pathway are repressed under Mg-sufficient conditions. Thus, the D-substrates all behaved similarly, with the exception of the apparent lack of magnesium inhibition of D-mannose conversion to AA. This would suggest that D-mannose enters the AA biosynthetic pathway at a point other than the other D-sugars.

Figs. 2A and 2B represent some of the fates of glucose in plants. The first enzymatic step in this scheme which commits carbon to glycolysis is the conversion of fructose-6-P to fructose-1,6-diP by phosphofructokinase (PFK). This reaction is essentially irreversible, and leads to the well known TCA cycle and oxidative phosphorylation, with concomitant ATP and NADH/NADPH generation. PFK has an absolute requirement for magnesium. If magnesium is limiting, this reaction could slow and eventually stop, blocking the flow of carbon through glycolysis and beyond, and would result in cessation of cell division even in the presence of excess glucose. One would expect fructose-6-P to accumulate under these conditions, fueling AA synthesis by the pathway shown in Figs. 1 and 2.

Example 7

The following example shows the correlation in *Prototheca* between AA production and the activity levels of the enzymes in the AA pathway.

Phosphomannose isomerase (PMI) Assay

PMI activity was first assayed (See Fig. 1). Ten strains representing a range of AA productivities were grown according to the standard protocol to measure AA-synthesizing ability. Cells were harvested 96 hours into magnesium-limited incubation, washed and resuspended in buffer containing 50 mM Tris/10 mM $MgCl_2$, pH 7.5. The suspended cells were broken in a French press, spun at 30,000 x g for 30 minutes, and desalted through Sephadex G-25 (Pharmacia PD-10 columns). Reactions were carried out in the reverse direction by adding various volumes of extracts to solutions of Tris/Mg buffer containing 0.15 U phosphoglucose isomerase (EC 5.3.1.9), 0.5 U glucose-6-phosphate dehydrogenase (EC 1.1.1.49), and 1.0 mM NADP. Reactions were initiated by addition of 3 mM (final) mannose-6-phosphate. Final reaction volume was 1.0 mL. All components were dissolved in Tris/Mg buffer. Activities were taken as the change in A_{340}/min. From these activities was subtracted the activities measured in identical reaction mixtures lacking the M-6-P substrate. Specific activities were calculated by normalizing the activities for protein concentration in the reactions. Protein in the original extracts was determined by the method of Bradford, using a kit from Bio-Rad Laboratories (Hercules, CA). All enzymes and nucleotides were purchased from Sigma Chemical Co. (St. Louis, MO).

Phosphomannomutase (PMM) Assay

Phosphomannomutase was measured in a similar manner in the same strains, but these assay reaction mixtures also contained 0.25 mM glucose-1,6-diphosphate, 0.5 U commercially available PMI, and the reactions were started with the addition of 3.0 mM (final) mannose-1-phosphate rather than mannose-6-phosphate.

Phosphofructokinase (PFK) Assay

To shed light on the possibility that the enhancement of AA concentration in cultures which were limited for magnesium was due to a diversion of carbon from normal metabolism by a reduced activity of the first committed step in glycolysis (PFK) the strains were also assayed to confirm the presence of this enzyme activity. Cells were cultured, washed and broken as above. Extracts were centrifuged at 100,000 x g for 90 min before

desalting. Reactions were carried out in the forward direction by adding various volumes of extracts to solutions of Tris/Mg buffer containing 1.5 mM dithiothreitol, 0.86 U aldolase (EC 4.1.2.13), 1.4 U α-glycerophosphate dehydrogenase (EC 1.1.1.8), 14 U triosephosphate isomerase (EC 5.3.1.1), 0.11 mM NADH, and 1.0 mM ATP. Reactions were initiated by addition of 5 mM (final) fructose-6-phosphate. Final reaction volume was 1.0 mL. All components were dissolved in Tris/Mg buffer. Activities were taken as the change in A_{340}/min. From these activities were subtracted the activities measured in identical reaction mixtures lacking the F-6-P substrate. Specific activities were calculated by normalizing the activities for protein concentration in the reaction. Protein in the original extracts was determined as above.

GDP-D-mannose pyrophosphorylase (GMP) Assay

These same mutant strains were assayed for the next enzyme in the proposed pathway, GMP. Strains were grown both according to the standard Mg-limiting protocol (harvested 43-48 hours into magnesium-limited incubation) and in standard Mg-sufficient medium (harvesting all cells before glucose depletion). Washed cell pellets were resuspended in 50 mM phosphate buffer, pH 7.0, containing 20% (v/v) glycerol and 0.1 M sodium chloride (3 mL buffer/g wet cells), and broken in a French press. Crude extracts were spun at 15,000 x g for 15 minutes. Reactions were carried out in the forward direction by adding various volumes of extracts to solutions of 50 mM phosphate/4 mM $MgCl_2$ buffer, pH 7.0, containing 1 mM GTP. Reactions were initiated by addition of 1 mM (final) mannose-1-phosphate. Final reaction volume was 0.1 mL. Reaction mixtures were incubated at 30 C for 10 min, filtered through a 0.45 µm PVDF syringe filter, and analyzed for GDP-mannose by HPLC. A Supelcosil SAX1 column (4.6 x 250 mm) was used with a solvent gradient (1 mL/min) of: A - 6 mM potassium phosphate, pH 3.6; B - 500 mM potassium phosphate, pH 4.5. The gradient was: 0-3 min, 100% A; 3-10 min, 79% A; 10-15 min, 29% A. Column temperature was 30 C. Two assays that showed enzyme activity proportional to the amount of protein were averaged. Control no-substrate and no-extract reactions were also run. Specific activity was calculated by normalizing the activity for protein concentration in the reaction. Protein in the original extracts was determined as above.

GDP-D-mannose:GDP-L-galactose Epimerase Assay

Further tests measured the activities of the next enzyme in the proposed pathway, GDP-D-mannose:GDP-L-galactose epimerase. Strains were grown according to the standard protocol, harvested 43-48 hours into magnesium-limited incubation, washed, and resuspended in buffer containing 50 mM MOPS/5 mM EDTA, pH 7.2. Washed pellets were broken in a French press, and spun at 20,000 x g for 20 min. Protein determinations were made as above and a dilution series of each was made, ranging from 0.4 to 2.2 mg protein/mL. 50 μL aliquots of these dilutions were added to 10 μL aliquots of 6.3 mM GDP-D-mannose in which a portion of this substrate was universally labeled with ^{14}C in the mannose moiety. This substrate had an activity of 16 μCi/mL before dilution into the reaction mixture. Reactions were stopped after 10 min by transferring 20 μL of the mixture into microfuge tubes containing 20 μL of 250 mM trifluoroacetic acid (TFA) containing 1.0 g/L each D-mannose and L-galactose. These tubes were sealed and boiled for 10 min, cooled, spun for 60 sec in a Beckman Microfuge E, and 5 μL of each hydrolysate was spotted on 20 x 20 cm plastic-backed EM Science Silica gel 60 thin-layer chromatography plates (#5748/7), with 1 cm lanes created by scoring with a blunt stylus. After drying, plates were twice chromatographed for 2.5 hours in ethyl acetate:isopropanol:water, 65:22.3:12.7 (plates were dried between runs). Spots of free sugars were visualized by spraying dried plates with 0.5% p-anisaldehyde in a 62% ethanolic solution of 0.89 M sulfuric acid and 0.17 mM glacial acetic acid, and heating at 105 C for about 15 min. Spots of L-galactose and D-mannose were cut from the plates and counted in a scintillation counter (Beckman model 2800). For time-zero control counts, 16.7 μL of each extract dilution was added to 23.3 μL of the labeled substrate above, which had been diluted 1:7 with the TFA/mannose/galactose solution.

Table 11 summarizes the results of the five enzyme assays for the strains tested, along with their specific AA formations.

TABLE 11

Specific Enzyme Activities (mU)* of Selected Mutant *Prototheca* Strains

Strain	AA Specific Form, mg/g	PMI	PMM	PFK	GMP		Epimerase
					Mg-limited	Mg-sufficient	
UV164-6	78.4						0.79
EMS13-4	73.7	10.8	69.6	13.5	2.6	6.8	0.78
UV140-1	69.9						0.78
NA45-3	61.4						0.58
UV77-247	44.4						0.52
UV127-10	40.1	11.1	45.8	24.4	4.3	5.9	0.39
UV244-15	24.5	14.3	41.5		3.1	5.3	0.42
NA21-14	23.6	12.1	60.3	47.4	2.4	7.6	0.27
ATCC 75669	21.9						0.28
UV244-16	5.0	16.5	85.6		4.3	5.2	
SP2-3	2.0	17.7	47.0	64.5	2.0	7.5	0.03
UV218-1	0.4	15.9	72.1		2.7	7.0	0.83
UV213-1	0.1	19.7	47.7	32.6	3.2	6.7	0.60
UV82-21	0.0	14.6	70.6	30.4	4.1	7.5	0.15
UV244-1	0.0	18.2	51.1		5.5	12	0.15

Units: PMI and PMM, nmoles NADP reduced per min/mg protein; PFK, nmoles NADH oxidized per min/mg protein; GMP, nmoles GDP-D-mannose formed per min/mg protein; epimerase, nmoles GDP-L-galactose formed per min/mg protein.

The only enzyme which showed a strong correlation between activity and the ability to synthesize AA was the GDP-D-mannose:GDP-L-galactose epimerase. This correlation is depicted in Fig. 5. All of the strains which produced measurable amounts of AA had measurable amounts of epimerase activity. The converse was not true: four of the strains which synthesize little or no AA had significant epimerase activities. These strains are candidates for having mutations which affect enzymatic steps downstream from the epimerase. Since all of the strains tested can synthesize AA from L-galactose and L-galactono-γ-lactone (see Examples 4 and 5), the genetic lesion(s) in these four mutants must lie between GDP-L-galactose and free L-galactose.

Example 8

The next example shows the relationship between GDP-D-mannose:GDP-L-galactose epimerase activity and the degree of magnesium limitation in two strains, the original unmutagenized parent strain ATCC 75669, and one of the best AA producers, EMS13-4 (ATCC ______).

Four flasks of each strain were grown according to the standard protocol. One culture of each was harvested 24 hours into magnesium-limited incubation, and every 24 hours thereafter for a total of four days. One flask of each strain was also harvested 24 hours into magnesium sufficient incubation. All cultures had glucose remaining when harvested. Fig. 6 shows graphically the AA productivity and epimerase activity in EMS13-4 and ATCC 75669 as the cultures became Mg-limited. Epimerase activity in EMS13-4 was significantly greater than that in ATCC 75669 at all time points. There was also a concurrent rapid rise in both AA productivity and epimerase activity in EMS13-4 as the cultures became increasingly Mg-limited. While there was a moderate increase in AA productivity in ATCC 75669 as Mg became more limiting, there was no effect on epimerase activity.

Example 9

The following example shows the results of epimerase assays performed with extracts of two *E. coli* strains into which were cloned the *E. coli* gene for GDP-4-keto-6-deoxy-D-mannose epimerase/reductase.

The *E. coli* K12 *wca* gene cluster is responsible for cholanic acid production; *wcaG* encodes a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase.

The *E. coli wcaG* sequence (nucleotides 4 through 966 of SEQ ID NO:3) was amplified by PCR from *E. coli* W3110 genomic DNA using primers WG EcoRI 5 (5' TAGAATTCAGTAAACAACGAGTTTTTATTGCTGG 3'; SEQ ID NO:12) and WG XhoI 3 (5' AACTCGAGTTACCCCCAAAGCGGTCTTGATTC 3'; SEQ ID NO:13). The 973-bp PCR product was ligated into the vector pPCR-Script SK(+) (Stratagene, LaJolla, CA). The 973-bp ExoRII/XhoI fragment was moved from this plasmid into the ExoRII/XhoI sites of pGEX-5X-1 (Amersham Pharmacia Biotech, Piscataway, NJ), creating plasmid pSW67-1. Plasmid pGEX-5X-1 is a GST gene fusion vector which adds a 26-kDa GST moiety onto the N-terminal end of the protein of interest. *E. coli* BL21(DE3) was transformed with pSW67-1 and pGEX-5X-1, resulting in strains BL21(DE3)/pSW67-1 and BL21(DE3)/pGEX-5X-1.

The *E. coli wcaG* sequence (nucleotides 1 through 966 of SEQ ID NO:3) was also amplified by PCR from *E. coli* W3110 genomic DNA using primers WG EcoRI 5-2 (5' CTGGAGTCGAATTCATGAGTAAACAACGAG 3'; SEQ ID NO:14) and WG PstI 3

(5' AACTGCAGTTACCCCCGAAAGCGGTCTTGATTC 3'; SEQ ID NO:15). The 976-bp PCR product was ligated into a pPCR-Script (Stratagene). The 976-bp ExoRII/PstI fragment was moved from this plasmid into the ExoRII/PstI sites of expression vector pKK223-3 (Amersham Pharmacia Biotech), creating plasmid pSW75-2. *E. coli* JM105 was transformed with pKK223-3 and pSW75-2, resulting in strains JM105/pKK223-3 and JM105/pSW75-2.

All six strains were grown in duplicate at 37°C with shaking in 2X YTA medium until an optical density of 0.8-1.0 at 600 nm was reached (about three hours). 2X YTA contains 16 g/L tryptone, 10 g/L yeast extract, 5 g/L sodium chloride and 100 mg/L ampicillin. One of each culture was induced by adding isopropyl β-D-thiogalactopyranoside (IPTG) to 1 mM final concentration. All 12 cultures were incubated for an additional four hours, washed in 0.9% NaCl, and the cells were frozen at -80°C. Prior to pelleting the cells for preparation of extracts, a portion of each culture was used for a plasmid DNA miniprep to confirm the presence of the appropriate plasmids in these strains. A protein preparation of each culture was also run on SDS gels to confirm expression of a protein of the appropriate size where expected. Frozen pellets were thawed, resuspended in 2.5 mL MOPS/EDTA buffer, pH 7.2, broken in a French Press (10,000 psi), spun for 20 min at 20,000 x g, assayed for protein as above and diluted to 0.01, 0.1, 1.0 and 3 mg/mL protein.

Induction of the strain BL21(DE3)/pGEX-5X-1 resulted in high-level expression of a 26-kDa protein indicating the synthesis of the native GST protein. Induction of strain BL21(DE3)/pSW67-1 resulted in high-level expression of a 62-kDa protein, indicating the synthesis of the native GST protein (26K) fused to the *wcaG* gene product (36K). An aliquot of the fusion protein was treated with the protease Factor Xa (New England Biolabs, Beverly, MA), which cleaves near the GST/*wcaG* junction. Induction of the strain JM105/pSW75-2 resulted in high level expression of a 36-kDa protein, indicating the synthesis of the *wcaG* gene product. No such protein was detected in JM105/pKK223-3 (vector only).

Next, it was of interest to test extracts in the standard epimerase assay described in Example 7 to determine if any of the extracts containing the *wcaG* product could bring

about the conversion of GDP-D-mannose to GDP-L-galactose. The extracts to be assayed are:

BL21(DE3) Group

1. BL21(DE3) uninduced
2. BL21(DE3) induced with 1mM IPTG
3. BL21(DE3)/pGEX-5X-1 uninduced
4. BL21(DE3)/pGEX-5X-1 induced with 1mM IPTG
5. BL21(DE3)/pSW67-1 uninduced
6. BL21(DE3)/pSW67-1 induced with 1 mM IPTG; fusion protein intact
7. BL21(DE3)/pSW67-1 induced with 1 mM IPTG; GST moiety cleaved

JM105 Group

1. JM105 uninduced
2. JM105 induced with 1mM IPTG
3. JM105/pKK223-3 uninduced
4. JM105/pKK223-3 induced with 1 mM IPTG
5. JM105/pSW75-2 uninduced
6. JM105/pSW75-2 induced with 1 mM IPTG

Extracts 1 and 7 from the BL21(DE3) group and extracts 1 and 6 from the JM105 group were tested for GDP-D-mannose:GDP-L-galactose epimerase-like activity in a pilot experiment. In this initial experiment, no epimerase activity was detected in any of the extracts. At this time, such a result can be attributed to a number of possibilities. First, it is possible that the *wcaG* gene product is incapable of catalyzing the conversion of GDP-D-mannose to GDP-L-galactose, although this conclusion can not be reached until several other parameters are tested. Second, it is possible that under the assay conditions which are satisfactory to measure activity for the endogenous GDP-D-mannose:GDP-L-galactose epimerase, the *wcaG* gene product does not have GDP-D-mannose:GDP-L-galactose epimerase-like activity. Therefore, alternate conditions should be tested. Additionally, confirmation experiments should be performed to confirm the accuracy of the pilot conditions. Third, although the BL21(DE3) and the JM105 clones produce proteins of the expected size, the constructs have not been sequenced to confirm the proper coding sequence for the *wcaG* gene product and thereby rule out PCR or cloning errors which may render the *wcaG* gene product inactive. Fourth, the protein formed from the cloned sequence is full-length, but inactive, for example, due to incorrect tertiary structure (folding). Fifth, the gene is overexpressed, resulting in accumulation of insoluble and inactive protein products (inclusion bodies). Future experiments will attempt to

determine whether the constructs have or can be induced to have the ability to catalyze the conversion of GDP-D-mannose to GDP-L-galactose, and to use the sequences to isolate the endogenous GDP-D-mannose:GDP-L-galactose epimerase.

Table 12 provides the atomic coordinates for Brookhaven Protein Data Bank Accession Code 1bws:

TABLE 12

```
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TITLE    3 THE BIOSYNTHESIS OF GDP-L-FUCOSE
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COMPND   2 MOLECULE: GDP-4-KETO-6-DEOXY-D-MANNOSE EPIMERASE/REDUCTASE;
COMPND   3 CHAIN: A;
COMPND   4 ENGINEERED: YES;
COMPND   5 BIOLOGICAL_UNIT: HOMODIMER
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SOURCE   3 EXPRESSION_SYSTEM: ESCHERICHIA COLI
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EXPDTA    X-RAY DIFFRACTION
AUTHOR    DE M.RIZZITONETTIFLORA
REVDAT   1   13-JAN-99 1BWS    0
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JRNL        TITL   GDP-4-KETO-6-DEOXYD-MANNOSE EPIMERASE/REDUCTASE
JRNL        TITL 2 FROM ESCHERICHIA COLI, A KEY ENZYME IN THE
JRNL        TITL 3 BIOSYNTHESIS OF GDP-L-FUCOSE, DISPLAYS THE
JRNL        TITL 4 STRUCTURAL CHARACTERISTICS OF THE RED PROTEIN
JRNL        TITL 5 HOMOLOGY SUPERFAMILY
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REMARK 290        4555   Y,X,-Z
REMARK 290        5555   X-Y,-Y,1/3-Z
REMARK 290        6555   -X,Y-X,2/3-Z
REMARK 290
REMARK 290     WHERE NNN -> OPERATOR NUMBER
REMARK 290            MMM -> TRANSLATION VECTOR
REMARK 290
REMARK 290 CRYSTALLOGRAPHIC SYMMETRY TRANSFORMATIONS
REMARK 290 THE FOLLOWING TRANSFORMATIONS OPERATE ON THE ATOM/HETATM
REMARK 290 RECORDS IN THIS ENTRY TO PRODUCE CRYSTALLOGRAPHICALLY
REMARK 290 RELATED MOLECULES.
REMARK 290   SMTRY1   1  1.000000  0.000000  0.000000        0.00000
REMARK 290   SMTRY2   1  0.000000  1.000000  0.000000        0.00000
REMARK 290   SMTRY3   1  0.000000  0.000000  1.000000        0.00000
REMARK 290   SMTRY1   2 -0.500045 -0.865974  0.000000        0.00000
REMARK 290   SMTRY2   2  0.866077 -0.499955  0.000000        0.00000
REMARK 290   SMTRY3   2  0.000000  0.000000  1.000000       50.58553
REMARK 290   SMTRY1   3 -0.499955  0.865974  0.000000        0.00000
REMARK 290   SMTRY2   3 -0.866077 -0.500045  0.000000        0.00000
REMARK 290   SMTRY3   3  0.000000  0.000000  1.000000       25.29276
REMARK 290   SMTRY1   4 -0.500045  0.865922  0.000000        0.00000
REMARK 290   SMTRY2   4  0.866077  0.500045  0.000000        0.00000
REMARK 290   SMTRY3   4  0.000000  0.000000 -1.000000        0.00000
REMARK 290   SMTRY1   5  1.000000  0.000104  0.000000        0.00000
REMARK 290   SMTRY2   5  0.000000 -1.000000  0.000000        0.00000
REMARK 290   SMTRY3   5  0.000000  0.000000 -1.000000       25.29276
REMARK 290   SMTRY1   6 -0.499955 -0.866026  0.000000        0.00000
REMARK 290   SMTRY2   6 -0.866077  0.499955  0.000000        0.00000
REMARK 290   SMTRY3   6  0.000000  0.000000 -1.000000       50.58553
REMARK 290
REMARK 290 REMARK: NULL
REMARK 465
REMARK 465 MISSING RESIDUES
REMARK 465 THE FOLLOWING RESIDUES WERE NOT LOCATED IN THE
REMARK 465 EXPERIMENT. (M=MODEL NUMBER; RES=RESIDUE NAME; C=CHAIN
REMARK 465 IDENTIFIER; SSSEQ=SEQUENCE NUMBER; I=INSERTION CODE):
REMARK 465
REMARK 465   M RES C SSSEQI
REMARK 465     MET A     1
REMARK 465     SER A     2
REMARK 465     ASP A   317
REMARK 465     ARG A   318
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REMARK 465       PHE A    319
REMARK 465       ARG A    320
REMARK 465       GLY A    321
REMARK 800
REMARK 800 SITE
REMARK 800 SITE_IDENTIFIER: CAT
REMARK 800 SITE_DESCRIPTION:
REMARK 800 CATALYTIC RESIDUE
REMARK 800
REMARK 800 SITE_IDENTIFIER: CAT
REMARK 800 SITE_DESCRIPTION:
REMARK 800 CATALYTIC RESIDUE
REMARK 800
REMARK 800 SITE_IDENTIFIER: CAT
REMARK 800 SITE_DESCRIPTION:
REMARK 800 CATALYTIC RESIDUE
REMARK 800
DBREF  1BWS A    3   316  SWS    P32055    FCL_ECOLI
SEQRES   1 A  321  MET SER LYS GLN ARG VAL PHE ILE ALA GLY HIS ARG GLY
SEQRES   2 A  321  MET VAL GLY SER ALA ILE ARG ARG GLN LEU GLU GLN ARG
SEQRES   3 A  321  GLY ASP VAL GLU LEU VAL LEU ARG THR ARG ASP GLU LEU
SEQRES   4 A  321  ASN LEU LEU ASP SER ARG ALA VAL HIS ASP PHE PHE ALA
SEQRES   5 A  321  SER GLU ARG ILE ASP GLN VAL TYR LEU ALA ALA ALA LYS
SEQRES   6 A  321  VAL GLY GLY ILE VAL ALA ASN ASN THR TYR PRO ALA ASP
SEQRES   7 A  321  PHE ILE TYR GLN ASN MET MET ILE GLU SER ASN ILE ILE
SEQRES   8 A  321  HIS ALA ALA HIS GLN ASN ASP VAL ASN LYS LEU LEU PHE
SEQRES   9 A  321  LEU GLY SER SER CYS ILE TYR PRO LYS LEU ALA LYS GLN
SEQRES  10 A  321  PRO MET ALA GLU SER GLU LEU LEU GLN GLY THR LEU GLU
SEQRES  11 A  321  PRO THR ASN GLU PRO TYR ALA ILE ALA LYS ILE ALA GLY
SEQRES  12 A  321  ILE LYS LEU CYS GLU SER TYR ASN ARG GLN TYR GLY ARG
SEQRES  13 A  321  ASP TYR ARG SER VAL MET PRO THR ASN LEU TYR GLY PRO
SEQRES  14 A  321  HIS ASP ASN PHE HIS PRO SER ASN SER HIS VAL ILE PRO
SEQRES  15 A  321  ALA LEU LEU ARG ARG PHE HIS GLU ALA THR ALA GLN ASN
SEQRES  16 A  321  ALA PRO ASP VAL VAL VAL TRP GLY SER GLY THR PRO MET
SEQRES  17 A  321  ARG GLU PHE LEU HIS VAL ASP ASP MET ALA ALA ALA SER
SEQRES  18 A  321  ILE HIS VAL MET GLU LEU ALA HIS GLU VAL TRP LEU GLU
SEQRES  19 A  321  ASN THR GLN PRO MET LEU SER HIS ILE ASN VAL GLY THR
SEQRES  20 A  321  GLY VAL ASP CYS THR ILE ARG ASP VAL ALA GLN THR ILE
SEQRES  21 A  321  ALA LYS VAL VAL GLY TYR LYS GLY ARG VAL VAL PHE ASP
SEQRES  22 A  321  ALA SER LYS PRO ASP GLY THR PRO ARG LYS LEU LEU ASP
SEQRES  23 A  321  VAL THR ARG LEU HIS GLN LEU GLY TRP TYR HIS GLU ILE
SEQRES  24 A  321  SER LEU GLU ALA GLY LEU ALA SER THR TYR GLN TRP PHE
SEQRES  25 A  321  LEU GLU ASN GLN ASP ARG PHE ARG GLY
HET    NDP      1       0
HETNAM     NDP NICOTINAMIDE ADENINE DINUCLEOTIDE PHOSPHATE
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HETSYN      NDP NADP
FORMUL   2  NDP    C21 H23 N7 O17 P3 3-
FORMUL   3  HOH   *109(H2 O1)
HELIX    1   1 MET A   14  GLN A   25  1                                  12
HELIX    2   2 SER A   44  GLU A   54  1                                  11
HELIX    3   3 ILE A   69  THR A   74  1                                   6
HELIX    4   4 PRO A   76  ASN A   97  1                                  22
HELIX    5   5 SER A  108  ILE A  110  5                                   3
HELIX    6   6 GLU A  121  GLU A  123  5                                   3
HELIX    7   7 GLU A  134  TYR A  154  1                                  21
HELIX    8   8 VAL A  180  ALA A  193  1                                  14
HELIX    9   9 VAL A  214  GLU A  226  1                                  13
HELIX   10  10 HIS A  229  GLU A  234  1                                   6
HELIX   11  11 ILE A  253  VAL A  264  1                                  12
HELIX   12  12 THR A  288  GLN A  292  1                                   5
HELIX   13  13 LEU A  301  GLU A  314  1                                  14
SHEET    1   A 6 VAL A  29   VAL A  32   0
SHEET    2   A 6 GLN A   4   ALA A   9   1  N  GLN A   4    O  GLU A  30
SHEET    3   A 6 GLN A  58   LEU A  61   1  N  GLN A  58    O  PHE A   7
SHEET    4   A 6 LYS A 101   LEU A 105   1  N  LYS A 101    O  VAL A  59
SHEET    5   A 6 ASP A 157   PRO A 163   1  N  ASP A 157    O  LEU A 102
SHEET    6   A 6 ILE A 243   VAL A 245   1  N  ILE A 243    O  MET A 162
SHEET    1   B 2 ASN A 165   TYR A 167   0
SHEET    2   B 2 PHE A 211   HIS A 213   1  N  LEU A 212    O  ASN A 165
SHEET    1   C 2 ASP A 198   TRP A 202   0
SHEET    2   C 2 ARG A 269   ASP A 273   1  N  ARG A 269    O  VAL A 199
SITE     1 CAT  1 TYR   136
SITE     2 CAT  1 LYS   140
SITE     3 CAT  1 SER   107
CRYST1  104.200  104.200   75.880  90.00  90.00 120.00 P 32 2 1      6
ORIGX1      1.000000  0.000000  0.000000        0.00000
ORIGX2      0.000000  1.000000  0.000000        0.00000
ORIGX3      0.000000  0.000000  1.000000        0.00000
SCALE1      0.009597  0.005541  0.000000        0.00000
SCALE2      0.000000  0.011081  0.000000        0.00000
SCALE3      0.000000  0.000000  0.013179        0.00000
HETATM    1  O   HOH     1      55.652 -16.806  22.535  1.00  8.73           O
HETATM    2  O   HOH     3      58.494 -10.639  18.740  1.00 13.17           O
HETATM    3  O   HOH     4      58.230 -11.715  27.770  1.00 19.07           O
HETATM    4  O   HOH     5      57.252  -3.759  30.107  1.00 11.21           O
HETATM    5  O   HOH     6      58.298 -10.011  25.527  1.00 15.74           O
HETATM    6  O   HOH     7      49.321   6.583  38.815  1.00 19.33           O
HETATM    7  O   HOH     8      53.785  -4.262  22.464  1.00 10.94           O
HETATM    8  O   HOH    10      74.652   2.888   9.141  1.00 17.80           O
HETATM    9  O   HOH    11      49.761   0.826  32.896  1.00 22.02           O
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HETATM	10	O	HOH	12	55.530	-11.162	28.526	1.00	11.39	O
HETATM	11	O	HOH	13	75.027	7.034	27.353	1.00	16.30	O
HETATM	12	O	HOH	14	49.994	-2.314	11.032	1.00	21.33	O
HETATM	13	O	HOH	15	61.323	-8.959	29.657	1.00	22.84	O
HETATM	14	O	HOH	16	61.029	-11.560	29.131	1.00	21.24	O
HETATM	15	O	HOH	17	50.684	5.881	10.130	1.00	15.88	O
HETATM	16	O	HOH	18	64.506	-6.302	32.989	1.00	21.05	O
HETATM	17	O	HOH	19	57.856	-16.398	25.085	1.00	22.86	O
HETATM	18	O	HOH	20	38.979	26.536	19.070	1.00	21.08	O
HETATM	19	O	HOH	21	38.042	33.487	21.909	1.00	19.01	O
HETATM	20	O	HOH	24	38.172	35.775	20.827	1.00	33.46	O
HETATM	21	O	HOH	25	70.916	-11.128	15.244	1.00	31.37	O
HETATM	22	O	HOH	26	54.205	19.360	28.396	1.00	35.76	O
HETATM	23	O	HOH	27	50.436	2.654	16.783	1.00	12.25	O
HETATM	24	O	HOH	28	69.692	19.108	38.979	1.00	49.77	O
HETATM	25	O	HOH	29	56.432	-8.877	19.303	1.00	22.52	O
HETATM	26	O	HOH	30	60.832	3.415	42.349	1.00	17.39	O
HETATM	27	O	HOH	31	53.889	-12.706	29.764	1.00	22.40	O
HETATM	28	O	HOH	32	37.887	26.373	28.058	1.00	18.09	O
HETATM	29	O	HOH	33	49.201	11.173	26.867	1.00	33.95	O
HETATM	30	O	HOH	34	46.762	-0.278	31.394	1.00	20.63	O
HETATM	31	O	HOH	35	41.731	27.568	43.302	1.00	27.39	O
HETATM	32	O	HOH	36	66.827	11.202	28.929	1.00	13.23	O
HETATM	33	O	HOH	37	46.834	14.396	40.819	1.00	46.02	O
HETATM	34	O	HOH	38	61.342	1.064	43.868	1.00	26.68	O
HETATM	35	O	HOH	42	70.597	16.422	37.837	1.00	19.26	O
HETATM	36	O	HOH	44	72.275	-9.089	33.407	1.00	22.11	O
HETATM	37	O	HOH	45	42.685	34.461	33.955	1.00	17.32	O
HETATM	38	O	HOH	46	53.480	13.394	38.364	1.00	20.19	O
HETATM	39	O	HOH	47	56.085	21.757	44.744	1.00	33.50	O
HETATM	40	O	HOH	48	35.741	32.691	23.517	1.00	19.49	O
HETATM	41	O	HOH	49	40.458	36.700	34.312	1.00	34.53	O
HETATM	42	O	HOH	50	75.440	7.267	29.948	1.00	18.07	O
HETATM	43	O	HOH	51	47.476	18.347	20.851	1.00	34.16	O
HETATM	44	O	HOH	53	52.837	-16.344	19.587	1.00	25.92	O
HETATM	45	O	HOH	55	46.415	9.073	20.108	1.00	31.91	O
HETATM	46	O	HOH	57	45.912	35.170	36.133	1.00	35.55	O
HETATM	47	O	HOH	58	60.247	-2.880	41.919	1.00	16.85	O
HETATM	48	O	HOH	60	64.974	6.086	24.501	1.00	32.16	O
HETATM	49	O	HOH	61	52.103	4.683	4.978	1.00	35.72	O
HETATM	50	O	HOH	62	50.888	40.154	36.463	1.00	38.35	O
HETATM	51	O	HOH	63	44.373	31.233	37.336	1.00	20.07	O
HETATM	52	O	HOH	64	57.280	27.757	42.451	1.00	21.74	O
HETATM	53	O	HOH	65	58.409	23.769	45.517	1.00	58.42	O
HETATM	54	O	HOH	66	68.690	-11.764	35.335	1.00	57.07	O

HETATM	55	O	HOH	67	42.746	25.153	23.465	1.00	27.05	O
HETATM	56	O	HOH	68	53.638	-16.457	32.292	1.00	31.71	O
HETATM	57	O	HOH	69	33.390	41.716	31.408	1.00	29.92	O
HETATM	58	O	HOH	70	57.768	17.897	42.434	1.00	25.75	O
HETATM	59	O	HOH	71	75.647	9.164	11.766	1.00	35.13	O
HETATM	60	O	HOH	72	62.032	33.292	44.749	1.00	46.18	O
HETATM	61	O	HOH	73	47.310	14.312	34.285	1.00	31.18	O
HETATM	62	O	HOH	74	79.660	-3.947	15.913	1.00	34.63	O
HETATM	63	O	HOH	75	46.929	5.343	4.550	1.00	23.14	O
HETATM	64	O	HOH	76	73.475	12.039	28.412	1.00	27.26	O
HETATM	65	O	HOH	77	46.297	-6.982	30.032	1.00	43.41	O
HETATM	66	O	HOH	78	68.528	-3.422	40.869	1.00	38.47	O
HETATM	67	O	HOH	79	62.080	-1.448	42.803	1.00	24.60	O
HETATM	68	O	HOH	80	65.330	18.150	40.726	1.00	41.00	O
HETATM	69	O	HOH	81	51.775	16.128	37.607	1.00	25.11	O
HETATM	70	O	HOH	83	54.266	28.682	43.313	1.00	27.61	O
HETATM	71	O	HOH	85	73.291	-15.479	20.603	1.00	37.54	O
HETATM	72	O	HOH	86	34.760	21.479	28.544	1.00	43.87	O
HETATM	73	O	HOH	87	37.326	24.131	29.677	1.00	24.47	O
HETATM	74	O	HOH	88	65.168	20.148	6.735	1.00	26.10	O
HETATM	75	O	HOH	89	59.196	12.089	13.630	1.00	25.24	O
HETATM	76	O	HOH	91	66.576	-6.235	40.279	1.00	43.11	O
HETATM	77	O	HOH	93	37.339	29.394	25.515	1.00	27.56	O
HETATM	78	O	HOH	94	52.339	-17.014	42.271	1.00	48.96	O
HETATM	79	O	HOH	95	40.511	32.927	31.717	1.00	22.46	O
HETATM	80	O	HOH	96	78.580	13.121	34.138	1.00	27.98	O
HETATM	81	O	HOH	97	65.090	15.704	34.876	1.00	18.96	O
HETATM	82	O	HOH	99	84.562	2.951	27.181	1.00	35.92	O
HETATM	83	O	HOH	100	50.386	9.761	9.646	1.00	23.18	O
HETATM	84	O	HOH	101	67.649	-0.851	38.764	1.00	24.99	O
HETATM	85	O	HOH	102	44.001	4.293	34.315	1.00	31.13	O
HETATM	86	O	HOH	103	59.386	-5.071	26.211	1.00	29.10	O
HETATM	87	O	HOH	104	77.364	4.745	41.506	1.00	35.32	O
HETATM	88	O	HOH	105	59.034	21.201	32.414	1.00	23.43	O
HETATM	89	O	HOH	106	42.463	34.698	14.327	1.00	38.86	O
HETATM	90	O	HOH	107	70.217	14.292	20.864	1.00	42.39	O
HETATM	91	O	HOH	108	76.999	8.130	25.862	1.00	32.91	O
HETATM	92	O	HOH	109	49.766	29.937	22.173	1.00	42.52	O
HETATM	93	O	HOH	110	72.473	13.536	38.823	1.00	33.32	O
HETATM	94	O	HOH	111	64.328	-12.084	38.608	1.00	37.99	O
HETATM	95	O	HOH	112	60.161	16.382	42.682	1.00	35.68	O
HETATM	96	O	HOH	113	47.602	13.639	27.016	1.00	26.01	O
HETATM	97	O	HOH	115	64.606	11.644	40.107	1.00	30.33	O
HETATM	98	O	HOH	116	61.231	-15.137	27.255	1.00	38.76	O
HETATM	99	O	HOH	117	65.324	-11.223	35.098	1.00	30.45	O

HETATM	100	O	HOH		119	56.602	17.219	44.932	1.00	36.53	O
HETATM	101	O	HOH		120	37.564	19.860	23.135	1.00	31.27	O
HETATM	102	O	HOH		121	64.845	5.057	21.132	1.00	45.57	O
HETATM	103	O	HOH		123	63.391	16.801	26.898	1.00	38.46	O
HETATM	104	O	HOH		124	42.567	6.134	32.635	1.00	31.56	O
HETATM	105	O	HOH		125	72.485	13.236	35.059	1.00	29.61	O
HETATM	106	O	HOH		126	65.229	3.650	44.032	1.00	36.86	O
HETATM	107	O	HOH		127	37.089	7.148	31.083	1.00	39.58	O
HETATM	108	O	HOH		128	73.327	10.546	12.123	1.00	34.97	O
HETATM	109	O	HOH		129	74.450	10.299	26.598	1.00	30.80	O
HETATM	110	AO5*	NDP	A	1	67.524	13.055	26.692	1.00	36.42	O
HETATM	111	AC5*	NDP	A	1	68.089	12.297	25.614	1.00	9.30	C
HETATM	112	AC4*	NDP	A	1	69.601	12.124	25.858	1.00	27.73	C
HETATM	113	AO4*	NDP	A	1	70.193	11.258	24.848	1.00	22.87	O
HETATM	114	AC3*	NDP	A	1	70.484	13.390	25.873	1.00	17.83	C
HETATM	115	AO3*	NDP	A	1	71.192	13.436	27.066	1.00	16.11	O
HETATM	116	AC2*	NDP	A	1	71.373	13.220	24.626	1.00	11.46	C
HETATM	117	AO2*	NDP	A	1	72.623	13.886	24.655	1.00	31.96	O
HETATM	118	AC1*	NDP	A	1	71.510	11.702	24.656	1.00	19.02	C
HETATM	119	O3	NDP	A	1	65.336	13.590	26.129	1.00	20.59	O
HETATM	120	NO5*	NDP	A	1	63.536	11.943	26.448	1.00	28.99	O
HETATM	121	NC5*	NDP	A	1	64.328	10.843	25.957	1.00	24.89	C
HETATM	122	NC4*	NDP	A	1	63.467	9.646	25.686	1.00	31.79	C
HETATM	123	NO4*	NDP	A	1	62.837	9.337	26.908	1.00	28.82	O
HETATM	124	NC3*	NDP	A	1	62.340	9.837	24.665	1.00	11.50	C
HETATM	125	NO3*	NDP	A	1	62.891	9.402	23.461	1.00	28.60	O
HETATM	126	NC2*	NDP	A	1	61.152	8.996	25.138	1.00	28.11	C
HETATM	127	NO2*	NDP	A	1	60.881	7.662	24.715	1.00	24.30	O
HETATM	128	NC1*	NDP	A	1	61.547	8.875	26.580	1.00	35.35	C
HETATM	129	AP2*	NDP	A	1	73.104	15.069	23.823	1.00	32.96	P
HETATM	130	AOP1	NDP	A	1	74.500	15.308	24.308	1.00	37.84	O
HETATM	131	AOP2	NDP	A	1	72.797	14.925	22.348	1.00	36.66	O
HETATM	132	AOP3	NDP	A	1	72.163	16.217	23.958	1.00	31.97	O
HETATM	133	AP	NDP	A	1	66.660	14.257	26.393	1.00	26.17	XX
HETATM	134	AO1	NDP	A	1	66.886	14.795	25.047	1.00	15.31	XX
HETATM	135	AO2	NDP	A	1	66.439	15.207	27.521	1.00	34.39	XX
HETATM	136	AN9	NDP	A	1	71.820	11.224	23.353	1.00	13.63	XX
HETATM	137	AC8	NDP	A	1	71.104	11.316	22.200	1.00	12.41	XX
HETATM	138	AN7	NDP	A	1	71.758	10.835	21.161	1.00	15.71	XX
HETATM	139	AC5	NDP	A	1	72.933	10.313	21.710	1.00	16.17	XX
HETATM	140	AC6	NDP	A	1	74.053	9.657	21.140	1.00	31.35	XX
HETATM	141	AN6	NDP	A	1	74.165	9.464	19.819	1.00	12.59	XX
HETATM	142	AN1	NDP	A	1	75.078	9.280	21.942	1.00	17.56	XX
HETATM	143	AC2	NDP	A	1	74.971	9.578	23.251	1.00	15.44	XX
HETATM	144	AN3	NDP	A	1	74.027	10.302	23.889	1.00	24.82	XX

HETATM	145	AC4	NDP	A	1	73.036	10.653	23.047	1.00	17.48	XX
HETATM	146	NP	NDP	A	1	64.183	13.106	27.191	1.00	25.47	N
HETATM	147	NO1	NDP	A	1	63.142	14.169	27.253	1.00	28.69	N
HETATM	148	NO2	NDP	A	1	64.837	12.643	28.492	1.00	24.32	N
HETATM	149	NN1	NDP	A	1	60.598	9.775	27.109	1.00	23.63	N
HETATM	150	NC2	NDP	A	1	60.143	10.905	26.442	-99.00	78.36	N
HETATM	151	NC3	NDP	A	1	59.070	11.648	27.007	-99.00	100.00	N
HETATM	152	NC7	NDP	A	1	58.497	13.017	26.528	-99.00	100.00	N
HETATM	153	NO7	NDP	A	1	59.358	13.703	25.972	-99.00	100.00	N
HETATM	154	NN7	NDP	A	1	57.207	13.400	26.912	-99.00	84.38	N
HETATM	155	NC4	NDP	A	1	58.442	11.146	28.137	-99.00	100.00	N
HETATM	156	NC5	NDP	A	1	58.912	9.963	28.754	-99.00	100.00	N
HETATM	157	NC6	NDP	A	1	59.951	9.266	28.147	-99.00	100.00	N
ATOM	158	N	LYS	A	3	76.227	-5.632	44.315	1.00	61.49	N
ATOM	159	CA	LYS	A	3	76.152	-4.302	43.684	1.00	58.00	C
ATOM	160	C	LYS	A	3	75.985	-4.421	42.171	1.00	52.79	C
ATOM	161	O	LYS	A	3	76.921	-4.737	41.419	1.00	44.76	O
ATOM	162	CB	LYS	A	3	77.359	-3.417	44.030	1.00	59.74	C
ATOM	163	CG	LYS	A	3	77.011	-1.944	44.314	1.00	50.87	C
ATOM	164	CD	LYS	A	3	78.208	-1.161	44.894	1.00	61.21	C
ATOM	165	CE	LYS	A	3	77.855	-0.377	46.186	1.00	100.00	C
ATOM	166	NZ	LYS	A	3	78.857	-0.401	47.343	1.00	70.61	N
ATOM	167	N	GLN	A	4	74.746	-4.242	41.747	1.00	45.15	N
ATOM	168	CA	GLN	A	4	74.408	-4.326	40.347	1.00	37.18	C
ATOM	169	C	GLN	A	4	74.983	-3.166	39.561	1.00	34.93	C
ATOM	170	O	GLN	A	4	75.127	-2.050	40.087	1.00	28.48	O
ATOM	171	CB	GLN	A	4	72.915	-4.445	40.221	1.00	34.65	C
ATOM	172	CG	GLN	A	4	72.456	-5.854	40.584	1.00	31.82	C
ATOM	173	CD	GLN	A	4	72.570	-6.788	39.405	1.00	79.25	C
ATOM	174	OE1	GLN	A	4	72.165	-6.452	38.286	1.00	100.00	O
ATOM	175	NE2	GLN	A	4	73.206	-7.925	39.623	1.00	80.24	N
ATOM	176	N	ARG	A	5	75.475	-3.495	38.375	1.00	27.16	N
ATOM	177	CA	ARG	A	5	76.146	-2.546	37.483	1.00	39.16	C
ATOM	178	C	ARG	A	5	75.191	-2.018	36.433	1.00	38.22	C
ATOM	179	O	ARG	A	5	74.938	-2.698	35.438	1.00	32.44	O
ATOM	180	CB	ARG	A	5	77.398	-3.163	36.826	1.00	41.76	C
ATOM	181	CG	ARG	A	5	78.692	-2.954	37.663	1.00	37.34	C
ATOM	182	CD	ARG	A	5	80.015	-3.236	36.876	1.00	32.99	C
ATOM	183	NE	ARG	A	5	81.036	-2.203	37.125	1.00	25.71	N
ATOM	184	CZ	ARG	A	5	81.617	-1.488	36.169	1.00	32.53	C
ATOM	185	NH1	ARG	A	5	81.293	-1.704	34.904	1.00	40.07	N
ATOM	186	NH2	ARG	A	5	82.516	-0.551	36.474	1.00	100.00	N
ATOM	187	N	VAL	A	6	74.743	-0.773	36.659	1.00	32.08	N
ATOM	188	CA	VAL	A	6	73.715	-0.082	35.881	1.00	28.89	C
ATOM	189	C	VAL	A	6	74.161	1.021	34.897	1.00	29.37	C

ATOM	190	O	VAL	A	6	74.745	2.041	35.274	1.00	22.50	O
ATOM	191	CB	VAL	A	6	72.577	0.378	36.813	1.00	23.52	C
ATOM	192	CG1	VAL	A	6	71.366	0.960	36.006	1.00	20.29	C
ATOM	193	CG2	VAL	A	6	72.108	-0.852	37.644	1.00	18.45	C
ATOM	194	N	PHE	A	7	73.948	0.749	33.615	1.00	22.92	N
ATOM	195	CA	PHE	A	7	74.267	1.710	32.573	1.00	27.15	C
ATOM	196	C	PHE	A	7	72.975	2.423	32.192	1.00	20.24	C
ATOM	197	O	PHE	A	7	71.994	1.788	31.815	1.00	20.71	O
ATOM	198	CB	PHE	A	7	74.864	1.004	31.374	1.00	18.98	C
ATOM	199	CG	PHE	A	7	74.916	1.836	30.115	1.00	21.83	C
ATOM	200	CD1	PHE	A	7	75.521	3.087	30.108	1.00	19.36	C
ATOM	201	CD2	PHE	A	7	74.483	1.284	28.886	1.00	23.50	C
ATOM	202	CE1	PHE	A	7	75.614	3.828	28.902	1.00	27.52	C
ATOM	203	CE2	PHE	A	7	74.548	1.996	27.685	1.00	19.33	C
ATOM	204	CZ	PHE	A	7	75.128	3.255	27.673	1.00	18.59	C
ATOM	205	N	ILE	A	8	72.959	3.727	32.454	1.00	18.75	N
ATOM	206	CA	ILE	A	8	71.844	4.588	32.112	1.00	14.25	C
ATOM	207	C	ILE	A	8	72.337	5.351	30.909	1.00	11.22	C
ATOM	208	O	ILE	A	8	73.259	6.165	30.998	1.00	17.76	O
ATOM	209	CB	ILE	A	8	71.507	5.605	33.212	1.00	14.15	C
ATOM	210	CG1	ILE	A	8	71.356	4.949	34.582	1.00	8.24	C
ATOM	211	CG2	ILE	A	8	70.183	6.342	32.874	1.00	16.85	C
ATOM	212	CD1	ILE	A	8	71.091	5.961	35.707	1.00	10.32	C
ATOM	213	N	ALA	A	9	71.896	4.906	29.752	1.00	16.42	N
ATOM	214	CA	ALA	A	9	72.256	5.559	28.513	1.00	18.74	C
ATOM	215	C	ALA	A	9	71.530	6.913	28.511	1.00	28.45	C
ATOM	216	O	ALA	A	9	70.411	7.032	29.045	1.00	22.39	O
ATOM	217	CB	ALA	A	9	71.808	4.731	27.311	1.00	14.43	C
ATOM	218	N	GLY	A	10	72.199	7.922	27.940	1.00	20.06	N
ATOM	219	CA	GLY	A	10	71.706	9.284	27.911	1.00	18.62	C
ATOM	220	C	GLY	A	10	71.407	9.819	29.305	1.00	16.40	C
ATOM	221	O	GLY	A	10	70.379	10.448	29.481	1.00	17.36	O
ATOM	222	N	HIS	A	11	72.295	9.581	30.272	1.00	10.32	N
ATOM	223	CA	HIS	A	11	72.068	9.966	31.688	1.00	13.90	C
ATOM	224	C	HIS	A	11	72.008	11.504	31.916	1.00	21.52	C
ATOM	225	O	HIS	A	11	71.700	11.994	32.983	1.00	13.22	O
ATOM	226	CB	HIS	A	11	73.153	9.350	32.581	1.00	14.88	C
ATOM	227	CG	HIS	A	11	74.502	9.948	32.326	1.00	23.73	C
ATOM	228	ND1	HIS	A	11	75.239	9.648	31.197	1.00	24.90	N
ATOM	229	CD2	HIS	A	11	75.167	10.952	32.956	1.00	16.35	C
ATOM	230	CE1	HIS	A	11	76.317	10.407	31.170	1.00	22.54	C
ATOM	231	NE2	HIS	A	11	76.271	11.240	32.197	1.00	17.56	N
ATOM	232	N	ARG	A	12	72.310	12.288	30.908	1.00	22.31	N
ATOM	233	CA	ARG	A	12	72.147	13.693	31.122	1.00	18.90	C
ATOM	234	C	ARG	A	12	70.851	14.244	30.495	1.00	26.34	C

ATOM	235	O	ARG	A	12	70.572	15.426	30.604	1.00	25.37	O
ATOM	236	CB	ARG	A	12	73.352	14.418	30.587	1.00	25.93	C
ATOM	237	CG	ARG	A	12	74.582	13.943	31.279	1.00	53.87	C
ATOM	238	CD	ARG	A	12	75.757	14.619	30.699	1.00	32.53	C
ATOM	239	NE	ARG	A	12	76.359	15.576	31.605	1.00	69.90	N
ATOM	240	CZ	ARG	A	12	76.971	16.675	31.178	1.00	100.00	C
ATOM	241	NH1	ARG	A	12	77.001	16.948	29.867	1.00	100.00	N
ATOM	242	NH2	ARG	A	12	77.526	17.508	32.056	1.00	100.00	N
ATOM	243	N	GLY	A	13	70.078	13.420	29.800	1.00	18.25	N
ATOM	244	CA	GLY	A	13	68.802	13.904	29.258	1.00	16.50	C
ATOM	245	C	GLY	A	13	67.849	14.144	30.428	1.00	18.88	C
ATOM	246	O	GLY	A	13	68.202	13.902	31.624	1.00	14.04	O
ATOM	247	N	MET	A	14	66.653	14.632	30.103	1.00	16.00	N
ATOM	248	CA	MET	A	14	65.688	14.981	31.128	1.00	13.49	C
ATOM	249	C	MET	A	14	65.293	13.760	31.901	1.00	14.02	C
ATOM	250	O	MET	A	14	65.408	13.713	33.145	1.00	17.06	O
ATOM	251	CB	MET	A	14	64.442	15.605	30.524	1.00	11.57	C
ATOM	252	CG	MET	A	14	63.320	15.628	31.559	1.00	20.77	C
ATOM	253	SD	MET	A	14	61.926	16.766	31.110	1.00	29.16	S
ATOM	254	CE	MET	A	14	62.527	17.108	29.574	1.00	30.68	C
ATOM	255	N	VAL	A	15	64.798	12.769	31.158	1.00	25.23	N
ATOM	256	CA	VAL	A	15	64.439	11.468	31.738	1.00	20.90	C
ATOM	257	C	VAL	A	15	65.654	10.713	32.378	1.00	17.26	C
ATOM	258	O	VAL	A	15	65.590	10.239	33.524	1.00	18.41	O
ATOM	259	CB	VAL	A	15	63.752	10.550	30.680	1.00	23.25	C
ATOM	260	CG1	VAL	A	15	63.330	9.253	31.310	1.00	15.71	C
ATOM	261	CG2	VAL	A	15	62.528	11.193	30.183	1.00	13.40	C
ATOM	262	N	GLY	A	16	66.784	10.642	31.665	1.00	20.39	N
ATOM	263	CA	GLY	A	16	67.941	9.904	32.186	1.00	19.54	C
ATOM	264	C	GLY	A	16	68.522	10.432	33.492	1.00	29.29	C
ATOM	265	O	GLY	A	16	68.896	9.659	34.434	1.00	16.91	O
ATOM	266	N	SER	A	17	68.642	11.755	33.499	1.00	12.53	N
ATOM	267	CA	SER	A	17	69.154	12.460	34.650	1.00	21.93	C
ATOM	268	C	SER	A	17	68.209	12.214	35.818	1.00	13.35	C
ATOM	269	O	SER	A	17	68.677	11.957	36.915	1.00	24.19	O
ATOM	270	CB	SER	A	17	69.378	13.942	34.333	1.00	15.52	C
ATOM	271	OG	SER	A	17	68.153	14.619	34.372	1.00	22.95	O
ATOM	272	N	ALA	A	18	66.896	12.143	35.590	1.00	17.52	N
ATOM	273	CA	ALA	A	18	65.991	11.828	36.729	1.00	13.14	C
ATOM	274	C	ALA	A	18	66.220	10.393	37.307	1.00	19.29	C
ATOM	275	O	ALA	A	18	66.149	10.150	38.522	1.00	16.94	O
ATOM	276	CB	ALA	A	18	64.460	12.046	36.334	1.00	14.33	C
ATOM	277	N	ILE	A	19	66.484	9.432	36.430	1.00	20.80	N
ATOM	278	CA	ILE	A	19	66.705	8.078	36.900	1.00	18.08	C
ATOM	279	C	ILE	A	19	67.975	8.090	37.730	1.00	16.09	C

ATOM	280	O	ILE	A	19	68.018	7.530	38.820	1.00 20.73	O
ATOM	281	CB	ILE	A	19	66.804	7.079	35.710	1.00 17.58	C
ATOM	282	CG1	ILE	A	19	65.444	6.812	35.162	1.00 10.09	C
ATOM	283	CG2	ILE	A	19	67.309	5.666	36.133	1.00 21.60	C
ATOM	284	CD1	ILE	A	19	65.528	6.361	33.741	1.00 19.05	C
ATOM	285	N	ARG	A	20	68.984	8.771	37.198	1.00 18.13	N
ATOM	286	CA	ARG	A	20	70.286	8.897	37.836	1.00 20.25	C
ATOM	287	C	ARG	A	20	70.231	9.491	39.242	1.00 30.62	C
ATOM	288	O	ARG	A	20	70.957	9.091	40.129	1.00 33.00	O
ATOM	289	CB	ARG	A	20	71.201	9.743	36.957	1.00 11.71	C
ATOM	290	CG	ARG	A	20	72.610	9.781	37.449	1.00 23.79	C
ATOM	291	CD	ARG	A	20	72.881	11.107	38.060	1.00 36.76	C
ATOM	292	NE	ARG	A	20	74.297	11.443	38.062	1.00 48.34	N
ATOM	293	CZ	ARG	A	20	74.990	11.841	36.988	1.00100.00	C
ATOM	294	NH1	ARG	A	20	74.393	11.931	35.808	1.00100.00	N
ATOM	295	NH2	ARG	A	20	76.289	12.139	37.076	1.00100.00	N
ATOM	296	N	ARG	A	21	69.368	10.461	39.439	1.00 22.10	N
ATOM	297	CA	ARG	A	21	69.216	11.052	40.750	1.00 17.45	C
ATOM	298	C	ARG	A	21	68.721	10.007	41.730	1.00 26.71	C
ATOM	299	O	ARG	A	21	69.147	10.001	42.885	1.00 30.27	O
ATOM	300	CB	ARG	A	21	68.142	12.144	40.708	1.00 17.93	C
ATOM	301	CG	ARG	A	21	68.682	13.522	40.321	1.00 27.57	C
ATOM	302	CD	ARG	A	21	67.586	14.599	40.130	1.00 23.02	C
ATOM	303	NE	ARG	A	21	67.619	15.000	38.743	1.00 55.12	N
ATOM	304	CZ	ARG	A	21	66.538	15.103	37.995	1.00 10.55	C
ATOM	305	NH1	ARG	A	21	65.343	14.974	38.552	1.00 29.80	N
ATOM	306	NH2	ARG	A	21	66.665	15.435	36.715	1.00 61.45	N
ATOM	307	N	GLN	A	22	67.713	9.223	41.345	1.00 27.48	N
ATOM	308	CA	GLN	A	22	67.167	8.257	42.313	1.00 24.79	C
ATOM	309	C	GLN	A	22	68.137	7.127	42.547	1.00 31.37	C
ATOM	310	O	GLN	A	22	68.394	6.724	43.685	1.00 27.47	O
ATOM	311	CB	GLN	A	22	65.818	7.706	41.894	1.00 17.11	C
ATOM	312	CG	GLN	A	22	64.921	8.745	41.243	1.00 66.14	C
ATOM	313	CD	GLN	A	22	63.425	8.456	41.397	1.00 41.27	C
ATOM	314	OE1	GLN	A	22	63.002	7.329	41.762	1.00 29.34	O
ATOM	315	NE2	GLN	A	22	62.610	9.464	41.046	1.00 20.12	N
ATOM	316	N	LEU	A	23	68.697	6.652	41.448	1.00 27.99	N
ATOM	317	CA	LEU	A	23	69.649	5.575	41.500	1.00 24.48	C
ATOM	318	C	LEU	A	23	70.828	5.971	42.334	1.00 28.87	C
ATOM	319	O	LEU	A	23	71.288	5.218	43.165	1.00 30.79	O
ATOM	320	CB	LEU	A	23	70.036	5.107	40.089	1.00 22.72	C
ATOM	321	CG	LEU	A	23	68.966	4.072	39.658	1.00 26.16	C
ATOM	322	CD1	LEU	A	23	69.271	3.083	38.481	1.00 24.80	C
ATOM	323	CD2	LEU	A	23	68.427	3.284	40.835	1.00 22.91	C
ATOM	324	N	GLU	A	24	71.279	7.192	42.153	1.00 28.77	N

ATOM	325	CA	GLU	A	24	72.419	7.675	42.909	1.00	33.79	C
ATOM	326	C	GLU	A	24	72.363	7.388	44.412	1.00	35.94	C
ATOM	327	O	GLU	A	24	73.381	7.140	45.031	1.00	39.07	O
ATOM	328	CB	GLU	A	24	72.647	9.165	42.653	1.00	36.21	C
ATOM	329	CG	GLU	A	24	74.068	9.482	42.243	1.00	42.54	C
ATOM	330	CD	GLU	A	24	74.158	10.689	41.333	1.00	89.51	C
ATOM	331	OE1	GLU	A	24	73.386	11.663	41.549	1.00	43.21	O
ATOM	332	OE2	GLU	A	24	74.994	10.646	40.398	1.00	66.28	O
ATOM	333	N	GLN	A	25	71.182	7.422	45.000	1.00	45.70	N
ATOM	334	CA	GLN	A	25	71.039	7.152	46.432	1.00	47.57	C
ATOM	335	C	GLN	A	25	70.887	5.669	46.740	1.00	67.34	C
ATOM	336	O	GLN	A	25	70.285	5.286	47.726	1.00	74.06	O
ATOM	337	CB	GLN	A	25	69.783	7.842	46.905	1.00	51.85	C
ATOM	338	CG	GLN	A	25	69.500	9.084	46.109	1.00	44.91	C
ATOM	339	CD	GLN	A	25	68.419	9.913	46.742	1.00100.00		C
ATOM	340	OE1	GLN	A	25	68.271	9.947	47.972	1.00100.00		O
ATOM	341	NE2	GLN	A	25	67.624	10.602	45.911	1.00100.00		N
ATOM	342	N	ARG	A	26	71.322	4.831	45.825	1.00	75.37	N
ATOM	343	CA	ARG	A	26	71.182	3.407	46.026	1.00	74.87	C
ATOM	344	C	ARG	A	26	72.568	2.791	46.147	1.00	74.08	C
ATOM	345	O	ARG	A	26	73.440	2.997	45.289	1.00	77.00	O
ATOM	346	CB	ARG	A	26	70.390	2.790	44.885	1.00	52.44	C
ATOM	347	CG	ARG	A	26	68.916	2.927	45.070	1.00	43.51	C
ATOM	348	CD	ARG	A	26	68.428	1.752	45.864	1.00	40.70	C
ATOM	349	NE	ARG	A	26	67.200	1.176	45.338	1.00	42.33	N
ATOM	350	CZ	ARG	A	26	67.126	0.508	44.196	1.00	32.07	C
ATOM	351	NH1	ARG	A	26	68.215	0.324	43.486	1.00	44.02	N
ATOM	352	NH2	ARG	A	26	65.968	0.017	43.771	1.00	77.32	N
ATOM	353	N	GLY	A	27	72.778	2.114	47.266	1.00	46.30	N
ATOM	354	CA	GLY	A	27	74.060	1.531	47.549	1.00	46.82	C
ATOM	355	C	GLY	A	27	74.140	0.165	46.923	1.00	55.45	C
ATOM	356	O	GLY	A	27	75.204	-0.453	46.877	1.00	64.43	O
ATOM	357	N	ASP	A	28	73.017	-0.315	46.428	1.00	40.98	N
ATOM	358	CA	ASP	A	28	73.016	-1.647	45.861	1.00	40.35	C
ATOM	359	C	ASP	A	28	73.266	-1.536	44.400	1.00	39.55	C
ATOM	360	O	ASP	A	28	73.109	-2.518	43.654	1.00	48.80	O
ATOM	361	CB	ASP	A	28	71.680	-2.335	46.127	1.00	47.80	C
ATOM	362	CG	ASP	A	28	70.503	-1.373	46.064	1.00	35.34	C
ATOM	363	OD1	ASP	A	28	70.705	-0.140	46.095	1.00	39.23	O
ATOM	364	OD2	ASP	A	28	69.383	-1.870	45.872	1.00	69.86	O
ATOM	365	N	VAL	A	29	73.651	-0.329	43.996	1.00	31.03	N
ATOM	366	CA	VAL	A	29	73.881	-0.050	42.591	1.00	28.44	C
ATOM	367	C	VAL	A	29	75.166	0.676	42.281	1.00	28.00	C
ATOM	368	O	VAL	A	29	75.505	1.699	42.892	1.00	34.83	O
ATOM	369	CB	VAL	A	29	72.696	0.760	42.000	1.00	30.68	C

ATOM	370	CG1	VAL	A	29	72.935	1.088	40.549	1.00	23.65	C
ATOM	371	CG2	VAL	A	29	71.416	-0.028	42.156	1.00	27.95	C
ATOM	372	N	GLU	A	30	75.824	0.219	41.230	1.00	30.76	N
ATOM	373	CA	GLU	A	30	76.995	0.924	40.736	1.00	28.38	C
ATOM	374	C	GLU	A	30	76.678	1.471	39.332	1.00	31.03	C
ATOM	375	O	GLU	A	30	76.368	0.720	38.397	1.00	26.64	O
ATOM	376	CB	GLU	A	30	78.199	0.006	40.722	1.00	31.84	C
ATOM	377	CG	GLU	A	30	79.355	0.539	41.533	1.00	89.26	C
ATOM	378	CD	GLU	A	30	80.667	0.264	40.858	1.00	100.00	C
ATOM	379	OE1	GLU	A	30	81.082	-0.922	40.872	1.00	88.94	O
ATOM	380	OE2	GLU	A	30	81.202	1.206	40.219	1.00	100.00	O
ATOM	381	N	LEU	A	31	76.665	2.789	39.207	1.00	22.24	N
ATOM	382	CA	LEU	A	31	76.269	3.391	37.945	1.00	29.37	C
ATOM	383	C	LEU	A	31	77.404	3.507	36.941	1.00	25.79	C
ATOM	384	O	LEU	A	31	78.485	3.969	37.256	1.00	29.41	O
ATOM	385	CB	LEU	A	31	75.632	4.760	38.191	1.00	30.20	C
ATOM	386	CG	LEU	A	31	74.329	4.763	38.994	1.00	29.37	C
ATOM	387	CD1	LEU	A	31	73.841	6.143	39.240	1.00	23.43	C
ATOM	388	CD2	LEU	A	31	73.275	3.962	38.281	1.00	23.04	C
ATOM	389	N	VAL	A	32	77.146	3.100	35.711	1.00	21.94	N
ATOM	390	CA	VAL	A	32	78.143	3.265	34.685	1.00	25.48	C
ATOM	391	C	VAL	A	32	77.535	4.242	33.669	1.00	38.76	C
ATOM	392	O	VAL	A	32	76.429	3.999	33.180	1.00	29.70	O
ATOM	393	CB	VAL	A	32	78.517	1.902	34.055	1.00	34.25	C
ATOM	394	CG1	VAL	A	32	79.587	2.079	32.970	1.00	30.56	C
ATOM	395	CG2	VAL	A	32	79.003	0.950	35.139	1.00	25.27	C
ATOM	396	N	LEU	A	33	78.219	5.375	33.457	1.00	30.19	N
ATOM	397	CA	LEU	A	33	77.732	6.463	32.621	1.00	22.71	C
ATOM	398	C	LEU	A	33	78.727	6.979	31.645	1.00	29.55	C
ATOM	399	O	LEU	A	33	79.896	7.152	31.988	1.00	30.09	O
ATOM	400	CB	LEU	A	33	77.423	7.635	33.514	1.00	19.75	C
ATOM	401	CG	LEU	A	33	76.729	7.200	34.779	1.00	19.38	C
ATOM	402	CD1	LEU	A	33	76.814	8.344	35.762	1.00	27.24	C
ATOM	403	CD2	LEU	A	33	75.271	6.913	34.444	1.00	22.07	C
ATOM	404	N	ARG	A	34	78.239	7.421	30.496	1.00	15.09	N
ATOM	405	CA	ARG	A	34	79.154	8.008	29.541	1.00	26.04	C
ATOM	406	C	ARG	A	34	78.469	9.173	28.916	1.00	36.57	C
ATOM	407	O	ARG	A	34	77.288	9.130	28.651	1.00	38.59	O
ATOM	408	CB	ARG	A	34	79.486	7.048	28.398	1.00	22.89	C
ATOM	409	CG	ARG	A	34	80.579	6.081	28.706	1.00	23.29	C
ATOM	410	CD	ARG	A	34	81.370	6.575	29.860	1.00	52.06	C
ATOM	411	NE	ARG	A	34	81.783	5.458	30.711	1.00	80.25	N
ATOM	412	CZ	ARG	A	34	82.646	4.530	30.323	1.00	41.94	C
ATOM	413	NH1	ARG	A	34	83.173	4.596	29.104	1.00	53.02	N
ATOM	414	NH2	ARG	A	34	82.983	3.547	31.148	1.00	25.56	N

ATOM	415	N	THR	A	35	79.248	10.156	28.539	1.00	31.58	N
ATOM	416	CA	THR	A	35	78.703	11.282	27.833	1.00	29.33	C
ATOM	417	C	THR	A	35	78.719	10.951	26.340	1.00	32.53	C
ATOM	418	O	THR	A	35	79.350	9.944	25.962	1.00	28.08	O
ATOM	419	CB	THR	A	35	79.527	12.527	28.145	1.00	37.49	C
ATOM	420	OG1	THR	A	35	80.844	12.429	27.560	1.00	31.91	O
ATOM	421	CG2	THR	A	35	79.627	12.642	29.651	1.00	19.38	C
ATOM	422	N	ARG	A	36	78.032	11.780	25.529	1.00	30.02	N
ATOM	423	CA	ARG	A	36	78.002	11.639	24.056	1.00	29.37	C
ATOM	424	C	ARG	A	36	79.406	11.765	23.503	1.00	31.46	C
ATOM	425	O	ARG	A	36	79.772	11.012	22.591	1.00	36.56	O
ATOM	426	CB	ARG	A	36	77.054	12.650	23.354	1.00	37.34	C
ATOM	427	CG	ARG	A	36	76.937	12.465	21.846	-99.00	49.47	C
ATOM	428	CD	ARG	A	36	76.020	13.515	21.232	-99.00	63.09	C
ATOM	429	NE	ARG	A	36	75.528	13.124	19.915	-99.00	75.23	N
ATOM	430	CZ	ARG	A	36	74.381	13.549	19.391	-99.00	91.44	C
ATOM	431	NH1	ARG	A	36	73.605	14.375	20.079	-99.00	79.32	N
ATOM	432	NH2	ARG	A	36	74.009	13.144	18.185	-99.00	78.73	N
ATOM	433	N	ASP	A	37	80.217	12.677	24.063	1.00	41.30	N
ATOM	434	CA	ASP	A	37	81.606	12.710	23.601	1.00	44.91	C
ATOM	435	C	ASP	A	37	82.410	11.481	24.043	1.00	24.99	C
ATOM	436	O	ASP	A	37	83.211	10.978	23.261	1.00	42.22	O
ATOM	437	CB	ASP	A	37	82.347	14.048	23.718	-99.00	47.07	C
ATOM	438	CG	ASP	A	37	81.881	14.887	24.876	-99.00	62.99	C
ATOM	439	OD1	ASP	A	37	80.679	14.839	25.204	-99.00	64.45	O
ATOM	440	OD2	ASP	A	37	82.711	15.638	25.429	-99.00	69.84	O
ATOM	441	N	GLU	A	38	82.129	10.950	25.235	1.00	19.39	N
ATOM	442	CA	GLU	A	38	82.790	9.717	25.682	1.00	27.84	C
ATOM	443	C	GLU	A	38	82.203	8.527	24.901	1.00	37.14	C
ATOM	444	O	GLU	A	38	82.873	7.511	24.699	1.00	35.04	O
ATOM	445	CB	GLU	A	38	82.691	9.435	27.207	1.00	25.18	C
ATOM	446	CG	GLU	A	38	83.116	10.549	28.183	1.00	37.45	C
ATOM	447	CD	GLU	A	38	82.807	10.212	29.655	1.00	21.13	C
ATOM	448	OE1	GLU	A	38	81.623	9.997	30.014	1.00	55.97	O
ATOM	449	OE2	GLU	A	38	83.757	9.978	30.419	1.00	98.78	O
ATOM	450	N	LEU	A	39	80.948	8.610	24.478	1.00	25.52	N
ATOM	451	CA	LEU	A	39	80.440	7.483	23.739	1.00	18.17	C
ATOM	452	C	LEU	A	39	79.291	7.764	22.825	1.00	20.34	C
ATOM	453	O	LEU	A	39	78.152	7.810	23.259	1.00	26.35	O
ATOM	454	CB	LEU	A	39	80.123	6.313	24.657	1.00	14.56	C
ATOM	455	CG	LEU	A	39	79.410	5.075	24.058	1.00	19.52	C
ATOM	456	CD1	LEU	A	39	80.205	4.392	22.994	1.00	18.84	C
ATOM	457	CD2	LEU	A	39	78.890	4.051	25.084	1.00	17.41	C
ATOM	458	N	ASN	A	40	79.598	7.880	21.543	1.00	16.73	N
ATOM	459	CA	ASN	A	40	78.548	7.971	20.540	1.00	21.55	C

ATOM	460	C	ASN A	40	77.798	6.649	20.308	1.00	24.53	C
ATOM	461	O	ASN A	40	78.328	5.720	19.688	1.00	19.96	O
ATOM	462	CB	ASN A	40	79.130	8.367	19.216	1.00	18.45	C
ATOM	463	CG	ASN A	40	78.054	8.727	18.225	1.00	42.19	C
ATOM	464	OD1	ASN A	40	78.327	9.093	17.080	1.00	38.89	O
ATOM	465	ND2	ASN A	40	76.827	8.730	18.697	1.00	23.71	N
ATOM	466	N	LEU A	41	76.543	6.622	20.754	1.00	21.08	N
ATOM	467	CA	LEU A	41	75.649	5.465	20.650	1.00	15.03	C
ATOM	468	C	LEU A	41	75.225	5.068	19.213	1.00	18.22	C
ATOM	469	O	LEU A	41	74.681	3.971	18.980	1.00	15.72	O
ATOM	470	CB	LEU A	41	74.426	5.705	21.532	1.00	15.85	C
ATOM	471	CG	LEU A	41	74.822	6.029	22.974	1.00	21.90	C
ATOM	472	CD1	LEU A	41	73.604	6.413	23.749	1.00	20.59	C
ATOM	473	CD2	LEU A	41	75.481	4.796	23.609	1.00	17.97	C
ATOM	474	N	LEU A	42	75.542	5.916	18.238	1.00	12.45	N
ATOM	475	CA	LEU A	42	75.256	5.607	16.831	1.00	15.99	C
ATOM	476	C	LEU A	42	76.290	4.680	16.280	1.00	26.18	C
ATOM	477	O	LEU A	42	76.066	4.039	15.257	1.00	22.41	O
ATOM	478	CB	LEU A	42	75.282	6.873	15.984	1.00	17.85	C
ATOM	479	CG	LEU A	42	74.180	7.854	16.399	1.00	30.70	C
ATOM	480	CD1	LEU A	42	74.318	9.184	15.704	1.00	24.31	C
ATOM	481	CD2	LEU A	42	72.764	7.241	16.208	1.00	31.13	C
ATOM	482	N	ASP A	43	77.462	4.705	16.911	1.00	26.87	N
ATOM	483	CA	ASP A	43	78.579	3.875	16.486	1.00	19.29	C
ATOM	484	C	ASP A	43	78.583	2.519	17.163	1.00	13.33	C
ATOM	485	O	ASP A	43	79.051	2.348	18.297	1.00	18.75	O
ATOM	486	CB	ASP A	43	79.870	4.580	16.776	1.00	31.06	C
ATOM	487	CG	ASP A	43	81.083	3.758	16.380	1.00	30.68	C
ATOM	488	OD1	ASP A	43	80.971	2.551	16.082	1.00	32.36	O
ATOM	489	OD2	ASP A	43	82.187	4.308	16.499	1.00	37.83	O
ATOM	490	N	SER A	44	78.139	1.544	16.377	1.00	16.89	N
ATOM	491	CA	SER A	44	77.978	0.173	16.789	1.00	17.67	C
ATOM	492	C	SER A	44	79.237	-0.463	17.392	1.00	20.40	C
ATOM	493	O	SER A	44	79.206	-1.126	18.444	1.00	26.27	O
ATOM	494	CB	SER A	44	77.504	-0.617	15.581	1.00	13.85	C
ATOM	495	OG	SER A	44	76.800	-1.740	16.063	1.00	43.83	O
ATOM	496	N	ARG A	45	80.335	-0.301	16.682	1.00	15.63	N
ATOM	497	CA	ARG A	45	81.616	-0.788	17.154	1.00	19.94	C
ATOM	498	C	ARG A	45	81.910	-0.225	18.521	1.00	29.48	C
ATOM	499	O	ARG A	45	82.244	-0.937	19.457	1.00	27.65	O
ATOM	500	CB	ARG A	45	82.684	-0.261	16.203	1.00	27.46	C
ATOM	501	CG	ARG A	45	83.463	-1.338	15.495	1.00	92.03	C
ATOM	502	CD	ARG A	45	84.854	-1.418	16.077	1.00100.00		C
ATOM	503	NE	ARG A	45	85.636	-2.533	15.527	1.00100.00		N
ATOM	504	CZ	ARG A	45	86.092	-3.570	16.236	1.00100.00		C

ATOM	505	NH1	ARG	A	45	85.791	-3.695	17.547	1.00	100.00	N
ATOM	506	NH2	ARG	A	45	86.773	-4.544	15.642	1.00	100.00	N
ATOM	507	N	ALA	A	46	81.772	1.090	18.629	1.00	31.04	N
ATOM	508	CA	ALA	A	46	82.045	1.743	19.881	1.00	24.72	C
ATOM	509	C	ALA	A	46	81.111	1.176	20.899	1.00	17.73	C
ATOM	510	O	ALA	A	46	81.512	0.825	22.027	1.00	22.73	O
ATOM	511	CB	ALA	A	46	81.839	3.221	19.751	1.00	27.16	C
ATOM	512	N	VAL	A	47	79.835	1.119	20.531	1.00	17.54	N
ATOM	513	CA	VAL	A	47	78.878	0.608	21.508	1.00	21.41	C
ATOM	514	C	VAL	A	47	79.262	-0.812	21.914	1.00	30.25	C
ATOM	515	O	VAL	A	47	79.192	-1.202	23.097	1.00	15.85	O
ATOM	516	CB	VAL	A	47	77.470	0.668	20.989	1.00	18.59	C
ATOM	517	CG1	VAL	A	47	76.503	0.042	22.012	1.00	16.88	C
ATOM	518	CG2	VAL	A	47	77.115	2.096	20.756	1.00	16.28	C
ATOM	519	N	HIS	A	48	79.692	-1.585	20.920	1.00	21.00	N
ATOM	520	CA	HIS	A	48	80.028	-2.969	21.192	1.00	20.17	C
ATOM	521	C	HIS	A	48	81.268	-3.079	22.117	1.00	32.98	C
ATOM	522	O	HIS	A	48	81.289	-3.850	23.102	1.00	28.20	O
ATOM	523	CB	HIS	A	48	80.063	-3.801	19.855	1.00	14.93	C
ATOM	524	CG	HIS	A	48	78.686	-4.172	19.338	1.00	26.67	C
ATOM	525	ND1	HIS	A	48	78.085	-5.394	19.600	1.00	28.83	N
ATOM	526	CD2	HIS	A	48	77.758	-3.448	18.659	1.00	25.56	C
ATOM	527	CE1	HIS	A	48	76.887	-5.430	19.043	1.00	20.08	C
ATOM	528	NE2	HIS	A	48	76.660	-4.260	18.475	1.00	25.22	N
ATOM	529	N	ASP	A	49	82.217	-2.170	21.902	1.00	22.62	N
ATOM	530	CA	ASP	A	49	83.455	-2.169	22.674	1.00	24.23	C
ATOM	531	C	ASP	A	49	83.171	-1.899	24.122	1.00	38.72	C
ATOM	532	O	ASP	A	49	83.708	-2.551	25.027	1.00	35.44	O
ATOM	533	CB	ASP	A	49	84.396	-1.112	22.127	1.00	30.29	C
ATOM	534	CG	ASP	A	49	84.991	-1.503	20.775	1.00	52.45	C
ATOM	535	OD1	ASP	A	49	85.007	-2.726	20.449	1.00	42.67	O
ATOM	536	OD2	ASP	A	49	85.416	-0.587	20.029	1.00	73.76	O
ATOM	537	N	PHE	A	50	82.294	-0.929	24.324	1.00	32.19	N
ATOM	538	CA	PHE	A	50	81.902	-0.550	25.649	1.00	29.76	C
ATOM	539	C	PHE	A	50	81.299	-1.765	26.359	1.00	30.31	C
ATOM	540	O	PHE	A	50	81.715	-2.124	27.449	1.00	29.22	O
ATOM	541	CB	PHE	A	50	80.892	0.610	25.576	1.00	23.82	C
ATOM	542	CG	PHE	A	50	80.137	0.843	26.859	1.00	19.13	C
ATOM	543	CD1	PHE	A	50	80.740	1.515	27.931	1.00	20.14	C
ATOM	544	CD2	PHE	A	50	78.835	0.360	27.018	1.00	13.99	C
ATOM	545	CE1	PHE	A	50	80.034	1.742	29.129	1.00	25.81	C
ATOM	546	CE2	PHE	A	50	78.114	0.553	28.212	1.00	22.84	C
ATOM	547	CZ	PHE	A	50	78.698	1.276	29.259	1.00	23.40	C
ATOM	548	N	PHE	A	51	80.280	-2.367	25.768	1.00	21.75	N
ATOM	549	CA	PHE	A	51	79.655	-3.451	26.457	1.00	22.61	C

ATOM	550	C	PHE	A	51	80.646	-4.603	26.612	1.00	34.01	C
ATOM	551	O	PHE	A	51	80.550	-5.401	27.590	1.00	25.28	O
ATOM	552	CB	PHE	A	51	78.389	-3.898	25.751	1.00	22.63	C
ATOM	553	CG	PHE	A	51	77.158	-3.140	26.170	1.00	27.58	C
ATOM	554	CD1	PHE	A	51	76.426	-3.525	27.280	1.00	21.78	C
ATOM	555	CD2	PHE	A	51	76.663	-2.100	25.380	1.00	19.55	C
ATOM	556	CE1	PHE	A	51	75.267	-2.796	27.662	1.00	28.34	C
ATOM	557	CE2	PHE	A	51	75.492	-1.403	25.734	1.00	14.47	C
ATOM	558	CZ	PHE	A	51	74.797	-1.744	26.878	1.00	14.55	C
ATOM	559	N	ALA	A	52	81.576	-4.706	25.659	1.00	26.43	N
ATOM	560	CA	ALA	A	52	82.587	-5.793	25.714	1.00	29.44	C
ATOM	561	C	ALA	A	52	83.687	-5.560	26.768	1.00	43.76	C
ATOM	562	O	ALA	A	52	84.502	-6.446	27.022	1.00	40.33	O
ATOM	563	CB	ALA	A	52	83.228	-6.049	24.344	1.00	24.25	C
ATOM	564	N	SER	A	53	83.702	-4.382	27.385	1.00	31.96	N
ATOM	565	CA	SER	A	53	84.705	-4.090	28.377	1.00	21.06	C
ATOM	566	C	SER	A	53	84.196	-3.625	29.709	1.00	26.41	C
ATOM	567	O	SER	A	53	84.985	-3.492	30.611	1.00	36.12	O
ATOM	568	CB	SER	A	53	85.709	-3.088	27.843	1.00	14.22	C
ATOM	569	OG	SER	A	53	85.140	-1.807	27.790	1.00	56.90	O
ATOM	570	N	GLU	A	54	82.892	-3.431	29.874	1.00	22.38	N
ATOM	571	CA	GLU	A	54	82.380	-2.893	31.139	1.00	17.27	C
ATOM	572	C	GLU	A	54	81.584	-3.735	32.118	1.00	26.32	C
ATOM	573	O	GLU	A	54	81.229	-3.281	33.191	1.00	37.43	O
ATOM	574	CB	GLU	A	54	81.677	-1.563	30.906	1.00	27.30	C
ATOM	575	CG	GLU	A	54	82.573	-0.543	30.262	1.00	44.77	C
ATOM	576	CD	GLU	A	54	83.669	-0.142	31.194	1.00	86.31	C
ATOM	577	OE1	GLU	A	54	83.392	-0.232	32.428	1.00	50.11	O
ATOM	578	OE2	GLU	A	54	84.785	0.198	30.692	1.00	50.99	O
ATOM	579	N	ARG	A	55	81.268	-4.971	31.804	1.00	29.63	N
ATOM	580	CA	ARG	A	55	80.636	-5.748	32.854	1.00	33.32	C
ATOM	581	C	ARG	A	55	79.347	-5.149	33.378	1.00	38.45	C
ATOM	582	O	ARG	A	55	79.214	-4.897	34.576	1.00	40.18	O
ATOM	583	CB	ARG	A	55	81.621	-5.875	34.045	1.00	57.61	C
ATOM	584	CG	ARG	A	55	82.666	-7.028	33.960	1.00	100.00	C
ATOM	585	CD	ARG	A	55	82.805	-7.805	35.305	1.00	100.00	C
ATOM	586	NE	ARG	A	55	82.838	-9.270	35.146	1.00	100.00	N
ATOM	587	CZ	ARG	A	55	83.206	-10.129	36.102	1.00	100.00	C
ATOM	588	NH1	ARG	A	55	83.583	-9.681	37.301	1.00	100.00	N
ATOM	589	NH2	ARG	A	55	83.208	-11.440	35.855	1.00	100.00	N
ATOM	590	N	ILE	A	56	78.367	-5.029	32.491	1.00	42.25	N
ATOM	591	CA	ILE	A	56	77.064	-4.434	32.794	1.00	25.49	C
ATOM	592	C	ILE	A	56	75.982	-5.474	33.244	1.00	20.18	C
ATOM	593	O	ILE	A	56	75.897	-6.579	32.704	1.00	24.74	O
ATOM	594	CB	ILE	A	56	76.672	-3.512	31.531	1.00	26.89	C

ATOM	595	CG1	ILE	A	56	77.643	-2.301	31.442	1.00	18.30	C
ATOM	596	CG2	ILE	A	56	75.214	-3.016	31.549	1.00	19.84	C
ATOM	597	CD1	ILE	A	56	77.998	-1.936	30.026	1.00	60.42	C
ATOM	598	N	ASP	A	57	75.166	-5.133	34.237	1.00	16.84	N
ATOM	599	CA	ASP	A	57	74.040	-5.999	34.630	1.00	16.33	C
ATOM	600	C	ASP	A	57	72.676	-5.451	34.123	1.00	28.40	C
ATOM	601	O	ASP	A	57	71.836	-6.198	33.657	1.00	25.50	O
ATOM	602	CB	ASP	A	57	74.009	-6.194	36.164	1.00	16.94	C
ATOM	603	CG	ASP	A	57	75.369	-6.720	36.703	1.00	34.27	C
ATOM	604	OD1	ASP	A	57	75.875	-7.729	36.141	1.00	31.76	O
ATOM	605	OD2	ASP	A	57	76.040	-6.007	37.499	1.00	28.36	O
ATOM	606	N	GLN	A	58	72.443	-4.152	34.220	1.00	28.91	N
ATOM	607	CA	GLN	A	58	71.183	-3.590	33.755	1.00	25.68	C
ATOM	608	C	GLN	A	58	71.425	-2.364	32.881	1.00	23.21	C
ATOM	609	O	GLN	A	58	72.403	-1.620	33.067	1.00	18.16	O
ATOM	610	CB	GLN	A	58	70.342	-3.151	34.946	1.00	33.14	C
ATOM	611	CG	GLN	A	58	69.798	-4.241	35.807	1.00	30.00	C
ATOM	612	CD	GLN	A	58	69.226	-3.712	37.105	1.00	27.18	C
ATOM	613	OE1	GLN	A	58	68.722	-2.601	37.161	1.00	31.20	O
ATOM	614	NE2	GLN	A	58	69.455	-4.436	38.186	1.00	16.89	N
ATOM	615	N	VAL	A	59	70.496	-2.138	31.961	1.00	18.35	N
ATOM	616	CA	VAL	A	59	70.562	-0.998	31.045	1.00	15.59	C
ATOM	617	C	VAL	A	59	69.238	-0.240	31.039	1.00	26.28	C
ATOM	618	O	VAL	A	59	68.178	-0.820	30.762	1.00	19.51	O
ATOM	619	CB	VAL	A	59	70.707	-1.456	29.601	1.00	15.32	C
ATOM	620	CG1	VAL	A	59	70.477	-0.274	28.649	1.00	11.93	C
ATOM	621	CG2	VAL	A	59	72.080	-2.111	29.364	1.00	15.83	C
ATOM	622	N	TYR	A	60	69.306	1.064	31.293	1.00	21.71	N
ATOM	623	CA	TYR	A	60	68.113	1.927	31.197	1.00	21.40	C
ATOM	624	C	TYR	A	60	68.289	2.756	29.928	1.00	18.69	C
ATOM	625	O	TYR	A	60	69.250	3.532	29.796	1.00	15.51	O
ATOM	626	CB	TYR	A	60	68.021	2.817	32.413	1.00	17.24	C
ATOM	627	CG	TYR	A	60	67.493	2.131	33.658	1.00	19.71	C
ATOM	628	CD1	TYR	A	60	68.345	1.583	34.586	1.00	21.14	C
ATOM	629	CD2	TYR	A	60	66.154	2.223	33.991	1.00	20.16	C
ATOM	630	CE1	TYR	A	60	67.835	1.080	35.794	1.00	19.11	C
ATOM	631	CE2	TYR	A	60	65.648	1.698	35.163	1.00	10.77	C
ATOM	632	CZ	TYR	A	60	66.476	1.094	36.054	1.00	20.07	C
ATOM	633	OH	TYR	A	60	65.921	0.585	37.248	1.00	16.04	O
ATOM	634	N	LEU	A	61	67.491	2.452	28.916	1.00	17.46	N
ATOM	635	CA	LEU	A	61	67.685	3.053	27.585	1.00	20.17	C
ATOM	636	C	LEU	A	61	67.003	4.412	27.409	1.00	23.36	C
ATOM	637	O	LEU	A	61	65.925	4.526	26.799	1.00	14.86	O
ATOM	638	CB	LEU	A	61	67.267	2.060	26.485	1.00	14.78	C
ATOM	639	CG	LEU	A	61	68.117	2.142	25.208	1.00	15.52	C

ATOM	640	CD1	LEU	A	61	67.815	1.010	24.109	1.00	7.75	C
ATOM	641	CD2	LEU	A	61	68.087	3.541	24.580	1.00	15.20	C
ATOM	642	N	ALA	A	62	67.656	5.434	27.956	1.00	20.35	N
ATOM	643	CA	ALA	A	62	67.120	6.784	27.963	1.00	18.55	C
ATOM	644	C	ALA	A	62	67.779	7.739	26.949	1.00	18.57	C
ATOM	645	O	ALA	A	62	67.455	8.924	26.920	1.00	24.31	O
ATOM	646	CB	ALA	A	62	67.071	7.377	29.439	1.00	11.69	C
ATOM	647	N	ALA	A	63	68.681	7.231	26.101	1.00	14.09	N
ATOM	648	CA	ALA	A	63	69.249	8.095	25.052	1.00	12.84	C
ATOM	649	C	ALA	A	63	68.310	8.005	23.877	1.00	27.00	C
ATOM	650	O	ALA	A	63	67.845	6.916	23.511	1.00	24.51	O
ATOM	651	CB	ALA	A	63	70.665	7.660	24.634	1.00	4.89	C
ATOM	652	N	ALA	A	64	68.076	9.148	23.262	1.00	21.05	N
ATOM	653	CA	ALA	A	64	67.202	9.286	22.086	1.00	13.50	C
ATOM	654	C	ALA	A	64	67.435	10.664	21.416	1.00	28.08	C
ATOM	655	O	ALA	A	64	67.987	11.600	22.021	1.00	26.63	O
ATOM	656	CB	ALA	A	64	65.642	9.171	22.518	1.00	7.63	C
ATOM	657	N	LYS	A	65	66.953	10.781	20.182	1.00	23.98	N
ATOM	658	CA	LYS	A	65	66.966	12.012	19.409	1.00	20.47	C
ATOM	659	C	LYS	A	65	65.488	12.443	19.551	1.00	24.37	C
ATOM	660	O	LYS	A	65	64.594	11.807	18.976	1.00	20.29	O
ATOM	661	CB	LYS	A	65	67.317	11.658	17.951	1.00	25.59	C
ATOM	662	CG	LYS	A	65	66.808	12.630	16.923	1.00	27.54	C
ATOM	663	CD	LYS	A	65	67.518	13.926	17.169	1.00	21.08	C
ATOM	664	CE	LYS	A	65	67.316	14.905	16.029	1.00	55.15	C
ATOM	665	NZ	LYS	A	65	67.876	16.263	16.392	1.00	81.63	N
ATOM	666	N	VAL	A	66	65.228	13.362	20.485	1.00	22.47	N
ATOM	667	CA	VAL	A	66	63.873	13.850	20.755	1.00	18.99	C
ATOM	668	C	VAL	A	66	63.711	15.343	20.394	1.00	31.44	C
ATOM	669	O	VAL	A	66	64.665	16.107	20.460	1.00	34.61	O
ATOM	670	CB	VAL	A	66	63.440	13.623	22.204	1.00	16.66	C
ATOM	671	CG1	VAL	A	66	64.269	12.623	22.869	1.00	15.01	C
ATOM	672	CG2	VAL	A	66	63.379	14.904	22.950	1.00	19.21	C
ATOM	673	N	GLY	A	67	62.514	15.755	19.994	1.00	18.03	N
ATOM	674	CA	GLY	A	67	62.298	17.149	19.614	1.00	14.90	C
ATOM	675	C	GLY	A	67	60.792	17.518	19.585	1.00	32.35	C
ATOM	676	O	GLY	A	67	59.922	16.666	19.888	1.00	18.88	O
ATOM	677	N	GLY	A	68	60.503	18.787	19.256	1.00	23.21	N
ATOM	678	CA	GLY	A	68	59.132	19.288	19.183	1.00	23.83	C
ATOM	679	C	GLY	A	68	58.540	19.137	17.771	1.00	19.31	C
ATOM	680	O	GLY	A	68	59.165	18.550	16.870	1.00	30.64	O
ATOM	681	N	ILE	A	69	57.343	19.684	17.588	1.00	15.20	N
ATOM	682	CA	ILE	A	69	56.595	19.632	16.317	1.00	16.80	C
ATOM	683	C	ILE	A	69	57.387	20.153	15.112	1.00	19.33	C
ATOM	684	O	ILE	A	69	57.425	19.519	14.061	1.00	14.66	O

ATOM	685	CB	ILE	A	69	55.257	20.432	16.480	1.00	30.11	C
ATOM	686	CG1	ILE	A	69	54.271	19.683	17.385	1.00	24.27	C
ATOM	687	CG2	ILE	A	69	54.610	20.749	15.181	1.00	47.53	C
ATOM	688	CD1	ILE	A	69	53.259	20.608	18.056	1.00	85.71	C
ATOM	689	N	VAL	A	70	58.010	21.327	15.269	1.00	23.03	N
ATOM	690	CA	VAL	A	70	58.797	21.913	14.183	1.00	19.34	C
ATOM	691	C	VAL	A	70	59.983	21.011	13.840	1.00	24.42	C
ATOM	692	O	VAL	A	70	60.335	20.829	12.662	1.00	24.14	O
ATOM	693	CB	VAL	A	70	59.304	23.404	14.467	1.00	21.37	C
ATOM	694	CG1	VAL	A	70	60.137	23.907	13.281	1.00	17.79	C
ATOM	695	CG2	VAL	A	70	58.136	24.410	14.678	1.00	15.74	C
ATOM	696	N	ALA	A	71	60.621	20.450	14.861	1.00	19.68	N
ATOM	697	CA	ALA	A	71	61.782	19.617	14.572	1.00	16.57	C
ATOM	698	C	ALA	A	71	61.427	18.289	13.910	1.00	23.36	C
ATOM	699	O	ALA	A	71	61.980	17.923	12.849	1.00	21.84	O
ATOM	700	CB	ALA	A	71	62.685	19.439	15.805	1.00	9.36	C
ATOM	701	N	ASN	A	72	60.463	17.598	14.511	1.00	16.80	N
ATOM	702	CA	ASN	A	72	59.998	16.357	13.923	1.00	18.84	C
ATOM	703	C	ASN	A	72	59.608	16.539	12.440	1.00	23.87	C
ATOM	704	O	ASN	A	72	59.919	15.696	11.593	1.00	21.52	O
ATOM	705	CB	ASN	A	72	58.835	15.806	14.738	1.00	8.60	C
ATOM	706	CG	ASN	A	72	59.309	15.013	15.911	1.00	23.75	C
ATOM	707	OD1	ASN	A	72	59.558	13.809	15.810	1.00	23.98	O
ATOM	708	ND2	ASN	A	72	59.572	15.701	16.996	1.00	9.96	N
ATOM	709	N	ASN	A	73	58.931	17.647	12.138	1.00	23.07	N
ATOM	710	CA	ASN	A	73	58.521	17.971	10.761	1.00	26.05	C
ATOM	711	C	ASN	A	73	59.665	18.454	9.817	1.00	26.95	C
ATOM	712	O	ASN	A	73	59.613	18.276	8.569	1.00	22.13	O
ATOM	713	CB	ASN	A	73	57.383	19.001	10.800	1.00	14.86	C
ATOM	714	CG	ASN	A	73	56.015	18.349	10.987	1.00	19.88	C
ATOM	715	OD1	ASN	A	73	55.620	17.468	10.217	1.00	27.02	O
ATOM	716	ND2	ASN	A	73	55.322	18.732	12.051	1.00	20.78	N
ATOM	717	N	THR	A	74	60.710	19.029	10.419	1.00	18.69	N
ATOM	718	CA	THR	A	74	61.845	19.540	9.657	1.00	10.07	C
ATOM	719	C	THR	A	74	62.968	18.548	9.375	1.00	21.00	C
ATOM	720	O	THR	A	74	63.537	18.561	8.289	1.00	11.75	O
ATOM	721	CB	THR	A	74	62.411	20.746	10.306	1.00	29.10	C
ATOM	722	OG1	THR	A	74	61.370	21.714	10.457	1.00	23.24	O
ATOM	723	CG2	THR	A	74	63.541	21.299	9.452	1.00	21.63	C
ATOM	724	N	TYR	A	75	63.230	17.636	10.310	1.00	17.10	N
ATOM	725	CA	TYR	A	75	64.267	16.620	10.112	1.00	9.07	C
ATOM	726	C	TYR	A	75	63.733	15.203	10.318	1.00	6.17	C
ATOM	727	O	TYR	A	75	64.143	14.542	11.267	1.00	15.58	O
ATOM	728	CB	TYR	A	75	65.302	16.825	11.188	1.00	11.89	C
ATOM	729	CG	TYR	A	75	65.779	18.234	11.252	1.00	27.12	C

ATOM	730	CD1	TYR	A	75	66.712	18.696	10.321	1.00	28.46	C
ATOM	731	CD2	TYR	A	75	65.234	19.151	12.173	1.00	24.83	C
ATOM	732	CE1	TYR	A	75	67.117	20.045	10.305	1.00	28.34	C
ATOM	733	CE2	TYR	A	75	65.652	20.523	12.180	1.00	21.00	C
ATOM	734	CZ	TYR	A	75	66.593	20.940	11.234	1.00	45.42	C
ATOM	735	OH	TYR	A	75	67.066	22.230	11.215	1.00	35.37	O
ATOM	736	N	PRO	A	76	62.759	14.775	9.532	1.00	13.30	N
ATOM	737	CA	PRO	A	76	62.185	13.438	9.742	1.00	14.64	C
ATOM	738	C	PRO	A	76	63.209	12.264	9.618	1.00	14.40	C
ATOM	739	O	PRO	A	76	63.157	11.335	10.409	1.00	20.54	O
ATOM	740	CB	PRO	A	76	61.055	13.366	8.709	1.00	7.83	C
ATOM	741	CG	PRO	A	76	61.447	14.388	7.617	1.00	12.61	C
ATOM	742	CD	PRO	A	76	62.068	15.504	8.455	1.00	11.18	C
ATOM	743	N	ALA	A	77	64.163	12.339	8.681	1.00	15.25	N
ATOM	744	CA	ALA	A	77	65.206	11.312	8.538	1.00	6.79	C
ATOM	745	C	ALA	A	77	66.053	11.166	9.820	1.00	17.22	C
ATOM	746	O	ALA	A	77	66.306	10.069	10.292	1.00	18.74	O
ATOM	747	CB	ALA	A	77	66.097	11.601	7.330	1.00	9.04	C
ATOM	748	N	ASP	A	78	66.466	12.267	10.424	1.00	10.92	N
ATOM	749	CA	ASP	A	78	67.256	12.191	11.659	1.00	11.87	C
ATOM	750	C	ASP	A	78	66.572	11.486	12.827	1.00	16.09	C
ATOM	751	O	ASP	A	78	67.212	10.741	13.601	1.00	18.07	O
ATOM	752	CB	ASP	A	78	67.578	13.609	12.088	1.00	19.16	C
ATOM	753	CG	ASP	A	78	68.424	14.325	11.068	1.00	26.82	C
ATOM	754	OD1	ASP	A	78	68.836	13.694	10.044	1.00	33.93	O
ATOM	755	OD2	ASP	A	78	68.673	15.514	11.316	1.00	32.06	O
ATOM	756	N	PHE	A	79	65.279	11.771	12.975	1.00	14.70	N
ATOM	757	CA	PHE	A	79	64.471	11.192	14.044	1.00	20.69	C
ATOM	758	C	PHE	A	79	64.224	9.707	13.876	1.00	20.22	C
ATOM	759	O	PHE	A	79	64.269	8.987	14.862	1.00	22.37	O
ATOM	760	CB	PHE	A	79	63.144	11.933	14.219	1.00	27.38	C
ATOM	761	CG	PHE	A	79	63.264	13.218	14.990	1.00	28.59	C
ATOM	762	CD1	PHE	A	79	63.137	13.230	16.386	1.00	27.49	C
ATOM	763	CD2	PHE	A	79	63.509	14.415	14.325	1.00	28.20	C
ATOM	764	CE1	PHE	A	79	63.281	14.413	17.109	1.00	21.76	C
ATOM	765	CE2	PHE	A	79	63.625	15.593	15.037	1.00	31.48	C
ATOM	766	CZ	PHE	A	79	63.509	15.582	16.439	1.00	26.31	C
ATOM	767	N	ILE	A	80	63.942	9.249	12.650	1.00	10.79	N
ATOM	768	CA	ILE	A	80	63.828	7.795	12.410	1.00	18.12	C
ATOM	769	C	ILE	A	80	65.197	7.052	12.432	1.00	10.97	C
ATOM	770	O	ILE	A	80	65.406	6.090	13.195	1.00	8.92	O
ATOM	771	CB	ILE	A	80	62.944	7.408	11.148	1.00	17.41	C
ATOM	772	CG1	ILE	A	80	62.651	5.886	11.105	1.00	10.16	C
ATOM	773	CG2	ILE	A	80	63.583	7.888	9.901	1.00	17.46	C
ATOM	774	CD1	ILE	A	80	61.722	5.410	9.980	1.00	7.30	C

ATOM	775	N	TYR	A	81	66.151	7.539	11.658	1.00	11.18	N
ATOM	776	CA	TYR	A	81	67.488	6.902	11.630	1.00	15.06	C
ATOM	777	C	TYR	A	81	68.237	6.782	12.959	1.00	16.83	C
ATOM	778	O	TYR	A	81	68.714	5.702	13.383	1.00	16.74	O
ATOM	779	CB	TYR	A	81	68.384	7.599	10.616	1.00	9.43	C
ATOM	780	CG	TYR	A	81	69.749	6.966	10.541	1.00	22.54	C
ATOM	781	CD1	TYR	A	81	69.963	5.824	9.747	1.00	22.37	C
ATOM	782	CD2	TYR	A	81	70.818	7.466	11.299	1.00	18.07	C
ATOM	783	CE1	TYR	A	81	71.202	5.163	9.746	1.00	15.02	C
ATOM	784	CE2	TYR	A	81	72.080	6.893	11.201	1.00	17.37	C
ATOM	785	CZ	TYR	A	81	72.255	5.698	10.472	1.00	24.27	C
ATOM	786	OH	TYR	A	81	73.491	5.063	10.409	1.00	19.57	O
ATOM	787	N	GLN	A	82	68.385	7.918	13.612	1.00	11.39	N
ATOM	788	CA	GLN	A	82	69.193	7.930	14.810	1.00	12.23	C
ATOM	789	C	GLN	A	82	68.544	7.089	15.834	1.00	14.18	C
ATOM	790	O	GLN	A	82	69.180	6.415	16.631	1.00	11.35	O
ATOM	791	CB	GLN	A	82	69.280	9.354	15.291	1.00	18.73	C
ATOM	792	CG	GLN	A	82	69.986	10.209	14.250	1.00	13.54	C
ATOM	793	CD	GLN	A	82	70.285	11.617	14.736	1.00	26.00	C
ATOM	794	OE1	GLN	A	82	70.410	11.850	15.927	1.00	22.99	O
ATOM	795	NE2	GLN	A	82	70.404	12.561	13.808	1.00	16.59	N
ATOM	796	N	ASN	A	83	67.235	7.181	15.869	1.00	11.35	N
ATOM	797	CA	ASN	A	83	66.549	6.408	16.860	1.00	13.71	C
ATOM	798	C	ASN	A	83	66.623	4.902	16.557	1.00	21.43	C
ATOM	799	O	ASN	A	83	66.831	4.101	17.463	1.00	12.10	O
ATOM	800	CB	ASN	A	83	65.132	6.945	17.074	1.00	13.51	C
ATOM	801	CG	ASN	A	83	65.131	8.245	17.871	1.00	28.91	C
ATOM	802	OD1	ASN	A	83	65.628	8.263	18.990	1.00	22.28	O
ATOM	803	ND2	ASN	A	83	64.756	9.354	17.237	1.00	20.17	N
ATOM	804	N	MET	A	84	66.592	4.517	15.290	1.00	15.63	N
ATOM	805	CA	MET	A	84	66.704	3.101	15.007	1.00	15.66	C
ATOM	806	C	MET	A	84	68.054	2.588	15.348	1.00	14.66	C
ATOM	807	O	MET	A	84	68.148	1.514	15.902	1.00	11.45	O
ATOM	808	CB	MET	A	84	66.418	2.815	13.563	1.00	17.59	C
ATOM	809	CG	MET	A	84	64.911	2.894	13.220	1.00	14.40	C
ATOM	810	SD	MET	A	84	64.638	2.811	11.387	1.00	15.99	S
ATOM	811	CE	MET	A	84	65.164	1.105	10.952	1.00	8.90	C
ATOM	812	N	MET	A	85	69.098	3.338	15.024	1.00	11.20	N
ATOM	813	CA	MET	A	85	70.468	2.879	15.321	1.00	11.67	C
ATOM	814	C	MET	A	85	70.779	2.831	16.774	1.00	13.04	C
ATOM	815	O	MET	A	85	71.359	1.893	17.265	1.00	15.26	O
ATOM	816	CB	MET	A	85	71.525	3.798	14.693	1.00	15.07	C
ATOM	817	CG	MET	A	85	71.530	3.726	13.173	1.00	32.01	C
ATOM	818	SD	MET	A	85	71.918	2.027	12.487	1.00	37.79	S
ATOM	819	CE	MET	A	85	73.379	1.801	13.320	1.00	15.94	C

ATOM	820	N	ILE	A	86	70.471	3.892	17.481	1.00	13.92	N
ATOM	821	CA	ILE	A	86	70.760	3.893	18.912	1.00	12.58	C
ATOM	822	C	ILE	A	86	70.159	2.662	19.591	1.00	21.61	C
ATOM	823	O	ILE	A	86	70.813	1.981	20.362	1.00	18.68	O
ATOM	824	CB	ILE	A	86	70.225	5.189	19.606	1.00	11.84	C
ATOM	825	CG1	ILE	A	86	70.978	6.429	19.119	1.00	19.78	C
ATOM	826	CG2	ILE	A	86	70.435	5.132	21.112	1.00	6.59	C
ATOM	827	CD1	ILE	A	86	70.505	7.694	19.772	1.00	20.37	C
ATOM	828	N	GLU	A	87	68.893	2.383	19.316	1.00	18.78	N
ATOM	829	CA	GLU	A	87	68.263	1.237	19.930	1.00	14.00	C
ATOM	830	C	GLU	A	87	68.797	-0.116	19.454	1.00	15.93	C
ATOM	831	O	GLU	A	87	69.017	-0.991	20.268	1.00	11.04	O
ATOM	832	CB	GLU	A	87	66.734	1.324	19.900	1.00	14.89	C
ATOM	833	CG	GLU	A	87	66.085	1.327	18.538	1.00	28.96	C
ATOM	834	CD	GLU	A	87	64.635	1.922	18.544	1.00	11.12	C
ATOM	835	OE1	GLU	A	87	64.307	2.801	19.376	1.00	25.46	O
ATOM	836	OE2	GLU	A	87	63.845	1.547	17.663	1.00	29.87	O
ATOM	837	N	SER	A	88	69.054	-0.259	18.155	1.00	16.18	N
ATOM	838	CA	SER	A	88	69.650	-1.482	17.569	1.00	19.52	C
ATOM	839	C	SER	A	88	71.029	-1.792	18.160	1.00	22.54	C
ATOM	840	O	SER	A	88	71.313	-2.929	18.592	1.00	13.80	O
ATOM	841	CB	SER	A	88	69.815	-1.326	16.023	1.00	14.61	C
ATOM	842	OG	SER	A	88	68.551	-1.201	15.355	1.00	15.41	O
ATOM	843	N	ASN	A	89	71.884	-0.773	18.143	1.00	22.63	N
ATOM	844	CA	ASN	A	89	73.227	-0.869	18.693	1.00	27.23	C
ATOM	845	C	ASN	A	89	73.195	-1.363	20.134	1.00	21.34	C
ATOM	846	O	ASN	A	89	73.795	-2.384	20.476	1.00	23.68	O
ATOM	847	CB	ASN	A	89	73.980	0.487	18.597	1.00	13.71	C
ATOM	848	CG	ASN	A	89	74.440	0.825	17.168	1.00	20.40	C
ATOM	849	OD1	ASN	A	89	74.305	-0.006	16.255	1.00	14.93	O
ATOM	850	ND2	ASN	A	89	74.937	2.067	16.960	1.00	13.32	N
ATOM	851	N	ILE	A	90	72.488	-0.646	20.979	1.00	16.55	N
ATOM	852	CA	ILE	A	90	72.437	-1.014	22.398	1.00	21.51	C
ATOM	853	C	ILE	A	90	71.876	-2.421	22.729	1.00	26.50	C
ATOM	854	O	ILE	A	90	72.384	-3.159	23.590	1.00	19.71	O
ATOM	855	CB	ILE	A	90	71.670	0.070	23.233	1.00	13.32	C
ATOM	856	CG1	ILE	A	90	72.539	1.299	23.401	1.00	11.05	C
ATOM	857	CG2	ILE	A	90	71.371	-0.445	24.637	1.00	7.54	C
ATOM	858	CD1	ILE	A	90	71.749	2.597	23.668	1.00	20.71	C
ATOM	859	N	ILE	A	91	70.755	-2.733	22.114	1.00	14.98	N
ATOM	860	CA	ILE	A	91	70.047	-3.953	22.442	1.00	21.33	C
ATOM	861	C	ILE	A	91	70.927	-5.098	21.994	1.00	26.27	C
ATOM	862	O	ILE	A	91	71.211	-6.011	22.751	1.00	26.56	O
ATOM	863	CB	ILE	A	91	68.556	-3.930	21.814	1.00	20.39	C
ATOM	864	CG1	ILE	A	91	67.692	-2.886	22.552	1.00	13.51	C

ATOM	865	CG2	ILE	A	91	67.841	-5.316	21.845	1.00	11.31	C
ATOM	866	CD1	ILE	A	91	66.320	-2.648	21.907	1.00	16.23	C
ATOM	867	N	HIS	A	92	71.446	-4.983	20.785	1.00	24.12	N
ATOM	868	CA	HIS	A	92	72.293	-6.015	20.243	1.00	26.71	C
ATOM	869	C	HIS	A	92	73.609	-6.251	21.071	1.00	29.30	C
ATOM	870	O	HIS	A	92	73.983	-7.366	21.443	1.00	18.58	O
ATOM	871	CB	HIS	A	92	72.561	-5.682	18.775	1.00	22.23	C
ATOM	872	CG	HIS	A	92	73.366	-6.720	18.077	1.00	26.32	C
ATOM	873	ND1	HIS	A	92	72.798	-7.711	17.307	1.00	27.19	N
ATOM	874	CD2	HIS	A	92	74.699	-6.978	18.106	1.00	21.95	C
ATOM	875	CE1	HIS	A	92	73.755	-8.487	16.826	1.00	23.66	C
ATOM	876	NE2	HIS	A	92	74.918	-8.062	17.296	1.00	17.36	N
ATOM	877	N	ALA	A	93	74.328	-5.187	21.333	1.00	15.66	N
ATOM	878	CA	ALA	A	93	75.530	-5.301	22.110	1.00	11.88	C
ATOM	879	C	ALA	A	93	75.222	-5.900	23.512	1.00	28.78	C
ATOM	880	O	ALA	A	93	75.912	-6.790	24.037	1.00	25.23	O
ATOM	881	CB	ALA	A	93	76.139	-3.959	22.221	1.00	6.30	C
ATOM	882	N	ALA	A	94	74.142	-5.442	24.113	1.00	18.82	N
ATOM	883	CA	ALA	A	94	73.777	-5.971	25.399	1.00	15.61	C
ATOM	884	C	ALA	A	94	73.593	-7.503	25.301	1.00	28.39	C
ATOM	885	O	ALA	A	94	74.133	-8.263	26.099	1.00	21.67	O
ATOM	886	CB	ALA	A	94	72.449	-5.279	25.911	1.00	18.46	C
ATOM	887	N	HIS	A	95	72.814	-7.966	24.329	1.00	26.35	N
ATOM	888	CA	HIS	A	95	72.551	-9.396	24.271	1.00	24.89	C
ATOM	889	C	HIS	A	95	73.845	-10.176	24.140	1.00	22.81	C
ATOM	890	O	HIS	A	95	74.077	-11.136	24.865	1.00	21.44	O
ATOM	891	CB	HIS	A	95	71.571	-9.778	23.129	1.00	22.39	C
ATOM	892	CG	HIS	A	95	71.554	-11.250	22.831	1.00	28.73	C
ATOM	893	ND1	HIS	A	95	70.979	-12.182	23.682	1.00	22.83	N
ATOM	894	CD2	HIS	A	95	72.159	-11.964	21.845	1.00	25.22	C
ATOM	895	CE1	HIS	A	95	71.171	-13.397	23.196	1.00	22.72	C
ATOM	896	NE2	HIS	A	95	71.911	-13.296	22.101	1.00	24.80	N
ATOM	897	N	GLN	A	96	74.709	-9.658	23.281	1.00	19.97	N
ATOM	898	CA	GLN	A	96	75.960	-10.299	22.917	1.00	22.27	C
ATOM	899	C	GLN	A	96	76.877	-10.353	24.086	1.00	26.58	C
ATOM	900	O	GLN	A	96	77.836	-11.093	24.088	1.00	24.17	O
ATOM	901	CB	GLN	A	96	76.642	-9.492	21.818	1.00	23.38	C
ATOM	902	CG	GLN	A	96	77.043	-10.299	20.596	1.00	61.06	C
ATOM	903	CD	GLN	A	96	78.033	-9.557	19.675	1.00	75.83	C
ATOM	904	OE1	GLN	A	96	78.999	-8.941	20.131	1.00	56.89	O
ATOM	905	NE2	GLN	A	96	77.815	-9.668	18.366	1.00	100.00	N
ATOM	906	N	ASN	A	97	76.652	-9.500	25.060	1.00	22.15	N
ATOM	907	CA	ASN	A	97	77.537	-9.536	26.208	1.00	14.74	C
ATOM	908	C	ASN	A	97	76.732	-10.022	27.387	1.00	29.78	C
ATOM	909	O	ASN	A	97	77.049	-9.762	28.564	1.00	27.09	O

ATOM	910	CB	ASN	A	97	78.241	-8.201	26.462	1.00	12.93	C
ATOM	911	CG	ASN	A	97	79.260	-7.897	25.407	1.00	24.91	C
ATOM	912	OD1	ASN	A	97	80.331	-8.518	25.375	1.00	57.17	O
ATOM	913	ND2	ASN	A	97	78.839	-7.135	24.392	1.00	34.88	N
ATOM	914	N	ASP	A	98	75.666	-10.732	27.055	1.00	27.98	N
ATOM	915	CA	ASP	A	98	74.907	-11.361	28.089	1.00	29.25	C
ATOM	916	C	ASP	A	98	74.400	-10.379	29.164	1.00	37.53	C
ATOM	917	O	ASP	A	98	74.505	-10.634	30.367	1.00	36.42	O
ATOM	918	CB	ASP	A	98	75.791	-12.450	28.700	1.00	36.37	C
ATOM	919	CG	ASP	A	98	75.016	-13.712	29.053	1.00	88.62	C
ATOM	920	OD1	ASP	A	98	73.775	-13.749	28.877	1.00	82.53	O
ATOM	921	OD2	ASP	A	98	75.656	-14.670	29.542	1.00	100.00	O
ATOM	922	N	VAL	A	99	73.879	-9.235	28.730	1.00	27.13	N
ATOM	923	CA	VAL	A	99	73.157	-8.351	29.635	1.00	21.57	C
ATOM	924	C	VAL	A	99	71.706	-8.868	29.530	1.00	16.15	C
ATOM	925	O	VAL	A	99	71.159	-9.088	28.422	1.00	19.47	O
ATOM	926	CB	VAL	A	99	73.264	-6.900	29.206	1.00	24.18	C
ATOM	927	CG1	VAL	A	99	72.517	-6.015	30.198	1.00	14.58	C
ATOM	928	CG2	VAL	A	99	74.720	-6.515	29.225	1.00	30.10	C
ATOM	929	N	ASN	A	100	71.149	-9.262	30.662	1.00	17.39	N
ATOM	930	CA	ASN	A	100	69.852	-9.925	30.613	1.00	25.77	C
ATOM	931	C	ASN	A	100	68.648	-9.034	30.910	1.00	24.95	C
ATOM	932	O	ASN	A	100	67.498	-9.377	30.582	1.00	20.88	O
ATOM	933	CB	ASN	A	100	69.846	-11.157	31.527	1.00	14.98	C
ATOM	934	CG	ASN	A	100	68.724	-12.112	31.180	1.00	20.38	C
ATOM	935	OD1	ASN	A	100	68.737	-12.709	30.100	1.00	29.59	O
ATOM	936	ND2	ASN	A	100	67.716	-12.240	32.076	1.00	16.35	N
ATOM	937	N	LYS	A	101	68.941	-7.923	31.584	1.00	17.91	N
ATOM	938	CA	LYS	A	101	67.970	-6.916	31.994	1.00	25.43	C
ATOM	939	C	LYS	A	101	68.107	-5.510	31.323	1.00	25.29	C
ATOM	940	O	LYS	A	101	69.151	-4.850	31.377	1.00	19.88	O
ATOM	941	CB	LYS	A	101	67.996	-6.807	33.521	1.00	29.28	C
ATOM	942	CG	LYS	A	101	67.464	-8.054	34.205	1.00	9.31	C
ATOM	943	CD	LYS	A	101	67.218	-7.719	35.668	1.00	38.93	C
ATOM	944	CE	LYS	A	101	66.206	-6.569	35.885	1.00	13.38	C
ATOM	945	NZ	LYS	A	101	64.750	-7.006	35.825	1.00	15.26	N
ATOM	946	N	LEU	A	102	67.013	-5.043	30.732	1.00	22.22	N
ATOM	947	CA	LEU	A	102	67.003	-3.744	30.092	1.00	15.40	C
ATOM	948	C	LEU	A	102	65.612	-3.115	30.156	1.00	18.55	C
ATOM	949	O	LEU	A	102	64.590	-3.811	30.102	1.00	18.92	O
ATOM	950	CB	LEU	A	102	67.465	-3.898	28.636	1.00	11.23	C
ATOM	951	CG	LEU	A	102	67.553	-2.711	27.651	1.00	15.51	C
ATOM	952	CD1	LEU	A	102	68.628	-2.985	26.559	1.00	9.65	C
ATOM	953	CD2	LEU	A	102	66.162	-2.407	26.995	1.00	13.10	C
ATOM	954	N	LEU	A	103	65.595	-1.798	30.318	1.00	17.05	N

ATOM	955	CA	LEU	A	103	64.356	-1.036	30.265	1.00	16.23	C
ATOM	956	C	LEU	A	103	64.346	-0.072	29.046	1.00	19.65	C
ATOM	957	O	LEU	A	103	65.215	0.789	28.875	1.00	19.68	O
ATOM	958	CB	LEU	A	103	64.099	-0.289	31.562	1.00	12.28	C
ATOM	959	CG	LEU	A	103	62.686	0.259	31.594	1.00	14.13	C
ATOM	960	CD1	LEU	A	103	61.645	-0.822	31.902	1.00	10.31	C
ATOM	961	CD2	LEU	A	103	62.646	1.360	32.601	1.00	12.30	C
ATOM	962	N	PHE	A	104	63.417	-0.333	28.140	1.00	16.41	N
ATOM	963	CA	PHE	A	104	63.215	0.486	26.956	1.00	18.32	C
ATOM	964	C	PHE	A	104	62.126	1.546	27.249	1.00	21.85	C
ATOM	965	O	PHE	A	104	61.168	1.271	27.992	1.00	18.36	O
ATOM	966	CB	PHE	A	104	62.796	-0.386	25.793	1.00	9.86	C
ATOM	967	CG	PHE	A	104	62.732	0.348	24.508	1.00	16.81	C
ATOM	968	CD1	PHE	A	104	63.894	0.714	23.840	1.00	25.04	C
ATOM	969	CD2	PHE	A	104	61.511	0.795	24.005	1.00	22.59	C
ATOM	970	CE1	PHE	A	104	63.836	1.448	22.619	1.00	31.26	C
ATOM	971	CE2	PHE	A	104	61.449	1.535	22.814	1.00	15.59	C
ATOM	972	CZ	PHE	A	104	62.625	1.895	22.139	1.00	11.67	C
ATOM	973	N	LEU	A	105	62.341	2.762	26.734	1.00	20.33	N
ATOM	974	CA	LEU	A	105	61.416	3.897	26.904	1.00	18.10	C
ATOM	975	C	LEU	A	105	60.711	4.237	25.634	1.00	17.04	C
ATOM	976	O	LEU	A	105	61.315	4.680	24.665	1.00	18.83	O
ATOM	977	CB	LEU	A	105	62.178	5.146	27.214	1.00	17.49	C
ATOM	978	CG	LEU	A	105	62.434	5.544	28.644	1.00	27.17	C
ATOM	979	CD1	LEU	A	105	62.630	4.349	29.574	1.00	19.16	C
ATOM	980	CD2	LEU	A	105	63.688	6.347	28.529	1.00	23.59	C
ATOM	981	N	GLY	A	106	59.407	4.153	25.652	1.00	20.66	N
ATOM	982	CA	GLY	A	106	58.679	4.536	24.455	1.00	21.03	C
ATOM	983	C	GLY	A	106	58.080	5.935	24.597	1.00	17.32	C
ATOM	984	O	GLY	A	106	58.690	6.858	25.113	1.00	26.89	O
ATOM	985	N	SER	A	107	56.831	6.047	24.219	1.00	22.05	N
ATOM	986	CA	SER	A	107	56.177	7.317	24.288	1.00	22.12	C
ATOM	987	C	SER	A	107	54.686	7.212	23.923	1.00	19.06	C
ATOM	988	O	SER	A	107	54.314	6.545	22.963	1.00	27.42	O
ATOM	989	CB	SER	A	107	56.882	8.232	23.300	1.00	20.99	C
ATOM	990	OG	SER	A	107	55.947	9.133	22.776	1.00	42.85	O
ATOM	991	N	SER	A	108	53.826	7.890	24.671	1.00	27.42	N
ATOM	992	CA	SER	A	108	52.382	7.947	24.339	1.00	26.43	C
ATOM	993	C	SER	A	108	52.144	8.259	22.842	1.00	30.97	C
ATOM	994	O	SER	A	108	51.242	7.709	22.217	1.00	33.46	O
ATOM	995	CB	SER	A	108	51.710	9.072	25.144	1.00	19.87	C
ATOM	996	OG	SER	A	108	52.495	10.266	25.071	1.00	70.88	O
ATOM	997	N	CYS	A	109	52.927	9.180	22.278	1.00	24.73	N
ATOM	998	CA	CYS	A	109	52.728	9.549	20.880	1.00	25.61	C
ATOM	999	C	CYS	A	109	52.970	8.482	19.815	1.00	21.29	C

ATOM	1000	O	CYS	A	109	52.967	8.737	18.623	1.00	31.31	O
ATOM	1001	CB	CYS	A	109	53.369	10.899	20.544	1.00	39.55	C
ATOM	1002	SG	CYS	A	109	55.153	11.077	20.847	1.00	49.24	S
ATOM	1003	N	ILE	A	110	53.101	7.264	20.258	1.00	18.31	N
ATOM	1004	CA	ILE	A	110	53.329	6.150	19.379	1.00	28.10	C
ATOM	1005	C	ILE	A	110	51.977	5.489	19.082	1.00	15.38	C
ATOM	1006	O	ILE	A	110	51.895	4.592	18.268	1.00	16.52	O
ATOM	1007	CB	ILE	A	110	54.154	5.153	20.206	1.00	40.45	C
ATOM	1008	CG1	ILE	A	110	55.604	5.510	20.136	1.00	39.02	C
ATOM	1009	CG2	ILE	A	110	53.879	3.715	19.875	1.00	61.33	C
ATOM	1010	CD1	ILE	A	110	56.429	4.338	20.549	1.00	82.74	C
ATOM	1011	N	TYR	A	111	50.951	5.842	19.854	1.00	14.91	N
ATOM	1012	CA	TYR	A	111	49.630	5.227	19.678	1.00	13.96	C
ATOM	1013	C	TYR	A	111	48.956	5.831	18.459	1.00	20.40	C
ATOM	1014	O	TYR	A	111	49.302	6.933	18.056	1.00	11.71	O
ATOM	1015	CB	TYR	A	111	48.763	5.468	20.921	1.00	9.63	C
ATOM	1016	CG	TYR	A	111	49.117	4.550	22.065	1.00	14.94	C
ATOM	1017	CD1	TYR	A	111	48.985	3.159	21.938	1.00	9.73	C
ATOM	1018	CD2	TYR	A	111	49.755	5.038	23.216	1.00	14.96	C
ATOM	1019	CE1	TYR	A	111	49.344	2.273	23.014	1.00	6.53	C
ATOM	1020	CE2	TYR	A	111	50.146	4.155	24.272	1.00	13.66	C
ATOM	1021	CZ	TYR	A	111	49.873	2.787	24.171	1.00	17.86	C
ATOM	1022	OH	TYR	A	111	50.266	1.927	25.157	1.00	11.37	O
ATOM	1023	N	PRO	A	112	47.974	5.145	17.872	1.00	22.56	N
ATOM	1024	CA	PRO	A	112	47.279	5.743	16.721	1.00	23.44	C
ATOM	1025	C	PRO	A	112	46.589	7.111	16.988	1.00	17.82	C
ATOM	1026	O	PRO	A	112	46.197	7.453	18.115	1.00	19.72	O
ATOM	1027	CB	PRO	A	112	46.290	4.644	16.252	1.00	15.69	C
ATOM	1028	CG	PRO	A	112	46.895	3.343	16.769	1.00	22.83	C
ATOM	1029	CD	PRO	A	112	47.593	3.733	18.086	1.00	16.10	C
ATOM	1030	N	LYS	A	113	46.418	7.866	15.915	1.00	19.48	N
ATOM	1031	CA	LYS	A	113	45.793	9.167	15.994	1.00	23.50	C
ATOM	1032	C	LYS	A	113	44.396	9.077	16.655	1.00	34.28	C
ATOM	1033	O	LYS	A	113	44.046	9.887	17.524	1.00	46.14	O
ATOM	1034	CB	LYS	A	113	45.675	9.735	14.593	1.00	30.04	C
ATOM	1035	CG	LYS	A	113	46.219	11.124	14.477	1.00	43.78	C
ATOM	1036	CD	LYS	A	113	45.381	11.941	13.515	1.00100.00		C
ATOM	1037	CE	LYS	A	113	44.361	12.836	14.250	1.00100.00		C
ATOM	1038	NZ	LYS	A	113	43.480	13.625	13.304	1.00100.00		N
ATOM	1039	N	LEU	A	114	43.591	8.103	16.250	1.00	26.33	N
ATOM	1040	CA	LEU	A	114	42.267	7.957	16.833	1.00	20.65	C
ATOM	1041	C	LEU	A	114	42.083	6.792	17.760	1.00	18.44	C
ATOM	1042	O	LEU	A	114	41.002	6.278	17.918	1.00	34.04	O
ATOM	1043	CB	LEU	A	114	41.194	8.002	15.780	1.00	24.37	C
ATOM	1044	CG	LEU	A	114	41.587	9.122	14.830	1.00	40.86	C

ATOM	1045	CD1	LEU	A	114	40.991	8.797	13.504	1.00	49.29	C
ATOM	1046	CD2	LEU	A	114	41.139	10.512	15.300	1.00	26.85	C
ATOM	1047	N	ALA	A	115	43.103	6.473	18.527	1.00	29.00	N
ATOM	1048	CA	ALA	A	115	42.920	5.446	19.528	1.00	25.66	C
ATOM	1049	C	ALA	A	115	41.722	5.727	20.454	1.00	28.76	C
ATOM	1050	O	ALA	A	115	41.364	6.855	20.682	1.00	24.12	O
ATOM	1051	CB	ALA	A	115	44.177	5.272	20.326	1.00	16.86	C
ATOM	1052	N	LYS	A	116	41.137	4.675	20.998	1.00	30.21	N
ATOM	1053	CA	LYS	A	116	40.036	4.792	21.928	1.00	25.85	C
ATOM	1054	C	LYS	A	116	40.668	5.248	23.195	1.00	14.18	C
ATOM	1055	O	LYS	A	116	41.750	4.781	23.535	1.00	23.51	O
ATOM	1056	CB	LYS	A	116	39.369	3.415	22.116	1.00	22.05	C
ATOM	1057	CG	LYS	A	116	39.053	3.032	23.524	1.00	55.38	C
ATOM	1058	CD	LYS	A	116	37.963	1.955	23.549	1.00	100.00	C
ATOM	1059	CE	LYS	A	116	37.120	1.953	24.835	1.00	100.00	C
ATOM	1060	NZ	LYS	A	116	35.767	1.310	24.630	1.00	100.00	N
ATOM	1061	N	GLN	A	117	40.021	6.208	23.856	1.00	18.23	N
ATOM	1062	CA	GLN	A	117	40.456	6.757	25.180	1.00	21.01	C
ATOM	1063	C	GLN	A	117	39.695	6.178	26.383	1.00	30.96	C
ATOM	1064	O	GLN	A	117	38.483	6.009	26.345	1.00	27.66	O
ATOM	1065	CB	GLN	A	117	40.215	8.263	25.179	1.00	11.32	C
ATOM	1066	CG	GLN	A	117	40.849	8.912	23.948	1.00	12.12	C
ATOM	1067	CD	GLN	A	117	42.404	8.823	23.954	1.00	24.10	C
ATOM	1068	OE1	GLN	A	117	43.041	8.628	22.896	1.00	47.88	O
ATOM	1069	NE2	GLN	A	117	43.001	8.953	25.131	1.00	14.24	N
ATOM	1070	N	PRO	A	118	40.374	5.992	27.499	1.00	30.02	N
ATOM	1071	CA	PRO	A	118	41.826	6.194	27.655	1.00	26.44	C
ATOM	1072	C	PRO	A	118	42.450	5.050	26.899	1.00	24.37	C
ATOM	1073	O	PRO	A	118	41.792	4.027	26.726	1.00	25.34	O
ATOM	1074	CB	PRO	A	118	42.055	5.994	29.167	1.00	23.89	C
ATOM	1075	CG	PRO	A	118	40.847	5.240	29.654	1.00	23.20	C
ATOM	1076	CD	PRO	A	118	39.695	5.519	28.709	1.00	15.79	C
ATOM	1077	N	MET	A	119	43.684	5.228	26.432	1.00	16.00	N
ATOM	1078	CA	MET	A	119	44.372	4.215	25.644	1.00	10.80	C
ATOM	1079	C	MET	A	119	45.062	3.083	26.444	1.00	23.61	C
ATOM	1080	O	MET	A	119	46.013	3.281	27.209	1.00	18.02	O
ATOM	1081	CB	MET	A	119	45.384	4.894	24.791	1.00	13.52	C
ATOM	1082	CG	MET	A	119	44.801	6.014	23.989	1.00	18.52	C
ATOM	1083	SD	MET	A	119	46.157	7.054	23.271	1.00	26.27	S
ATOM	1084	CE	MET	A	119	46.264	6.524	21.845	1.00	33.79	C
ATOM	1085	N	ALA	A	120	44.559	1.875	26.271	1.00	26.64	N
ATOM	1086	CA	ALA	A	120	45.177	0.712	26.884	1.00	29.17	C
ATOM	1087	C	ALA	A	120	46.356	0.308	25.984	1.00	23.21	C
ATOM	1088	O	ALA	A	120	46.439	0.759	24.833	1.00	20.19	O
ATOM	1089	CB	ALA	A	120	44.169	-0.419	26.944	1.00	26.02	C

ATOM	1090	N	GLU	A	121	47.238	-0.553	26.507	1.00	12.30	N
ATOM	1091	CA	GLU	A	121	48.427	-1.009	25.788	1.00	9.45	C
ATOM	1092	C	GLU	A	121	48.070	-1.697	24.450	1.00	11.68	C
ATOM	1093	O	GLU	A	121	48.828	-1.670	23.450	1.00	14.84	O
ATOM	1094	CB	GLU	A	121	49.321	-1.883	26.715	1.00	16.74	C
ATOM	1095	CG	GLU	A	121	50.132	-1.122	27.763	1.00	18.14	C
ATOM	1096	CD	GLU	A	121	49.458	-1.000	29.137	1.00	13.00	C
ATOM	1097	OE1	GLU	A	121	48.252	-1.294	29.276	1.00	20.79	O
ATOM	1098	OE2	GLU	A	121	50.123	-0.521	30.080	1.00	17.86	O
ATOM	1099	N	SER	A	122	46.887	-2.273	24.409	1.00	11.79	N
ATOM	1100	CA	SER	A	122	46.427	-2.977	23.218	1.00	12.16	C
ATOM	1101	C	SER	A	122	46.030	-2.058	22.100	1.00	11.70	C
ATOM	1102	O	SER	A	122	45.717	-2.529	21.010	1.00	13.91	O
ATOM	1103	CB	SER	A	122	45.186	-3.781	23.568	1.00	21.50	C
ATOM	1104	OG	SER	A	122	44.143	-2.908	23.976	1.00	28.52	O
ATOM	1105	N	GLU	A	123	46.041	-0.754	22.341	1.00	14.65	N
ATOM	1106	CA	GLU	A	123	45.783	0.202	21.243	1.00	17.15	C
ATOM	1107	C	GLU	A	123	46.959	0.313	20.240	1.00	11.48	C
ATOM	1108	O	GLU	A	123	46.821	0.844	19.141	1.00	11.19	O
ATOM	1109	CB	GLU	A	123	45.481	1.600	21.805	1.00	21.66	C
ATOM	1110	CG	GLU	A	123	44.127	1.694	22.523	1.00	24.68	C
ATOM	1111	CD	GLU	A	123	42.984	1.374	21.585	1.00	35.56	C
ATOM	1112	OE1	GLU	A	123	43.019	1.865	20.426	1.00	41.73	O
ATOM	1113	OE2	GLU	A	123	42.158	0.497	21.940	1.00100.00		O
ATOM	1114	N	LEU	A	124	48.134	-0.185	20.618	1.00	14.02	N
ATOM	1115	CA	LEU	A	124	49.296	-0.082	19.740	1.00	15.32	C
ATOM	1116	C	LEU	A	124	49.082	-0.754	18.458	1.00	17.76	C
ATOM	1117	O	LEU	A	124	48.752	-1.917	18.445	1.00	18.91	O
ATOM	1118	CB	LEU	A	124	50.564	-0.680	20.362	1.00	18.07	C
ATOM	1119	CG	LEU	A	124	51.922	-0.222	19.803	1.00	21.52	C
ATOM	1120	CD1	LEU	A	124	52.080	1.258	20.117	1.00	20.35	C
ATOM	1121	CD2	LEU	A	124	53.042	-0.919	20.550	1.00	14.07	C
ATOM	1122	N	LEU	A	125	49.514	-0.071	17.409	1.00	18.44	N
ATOM	1123	CA	LEU	A	125	49.445	-0.564	16.052	1.00	19.92	C
ATOM	1124	C	LEU	A	125	48.034	-0.754	15.509	1.00	25.56	C
ATOM	1125	O	LEU	A	125	47.854	-1.188	14.364	1.00	18.26	O
ATOM	1126	CB	LEU	A	125	50.355	-1.800	15.840	1.00	20.79	C
ATOM	1127	CG	LEU	A	125	51.890	-1.511	15.778	1.00	17.21	C
ATOM	1128	CD1	LEU	A	125	52.744	-2.649	16.316	1.00	19.95	C
ATOM	1129	CD2	LEU	A	125	52.334	-1.219	14.338	1.00	5.81	C
ATOM	1130	N	GLN	A	126	47.027	-0.327	16.276	1.00	21.97	N
ATOM	1131	CA	GLN	A	126	45.652	-0.504	15.790	1.00	19.97	C
ATOM	1132	C	GLN	A	126	45.213	0.447	14.724	1.00	28.31	C
ATOM	1133	O	GLN	A	126	44.076	0.391	14.293	1.00	47.49	O
ATOM	1134	CB	GLN	A	126	44.652	-0.404	16.911	1.00	19.87	C

ATOM	1135	CG	GLN	A	126	44.949	-1.312	18.048	1.00	18.39	C
ATOM	1136	CD	GLN	A	126	44.319	-2.626	17.835	1.00	66.80	C
ATOM	1137	OE1	GLN	A	126	44.064	-3.376	18.792	1.00	40.75	O
ATOM	1138	NE2	GLN	A	126	44.015	-2.952	16.565	1.00	71.74	N
ATOM	1139	N	GLY	A	127	46.080	1.330	14.270	1.00	28.29	N
ATOM	1140	CA	GLY	A	127	45.627	2.260	13.252	1.00	23.31	C
ATOM	1141	C	GLY	A	127	46.662	3.315	12.953	1.00	22.90	C
ATOM	1142	O	GLY	A	127	47.755	3.254	13.474	1.00	25.30	O
ATOM	1143	N	THR	A	128	46.311	4.219	12.046	1.00	19.51	N
ATOM	1144	CA	THR	A	128	47.149	5.314	11.588	1.00	22.12	C
ATOM	1145	C	THR	A	128	47.705	6.219	12.695	1.00	22.60	C
ATOM	1146	O	THR	A	128	47.061	6.461	13.731	1.00	18.58	O
ATOM	1147	CB	THR	A	128	46.392	6.182	10.544	1.00	35.98	C
ATOM	1148	OG1	THR	A	128	46.533	5.594	9.239	1.00	58.05	O
ATOM	1149	CG2	THR	A	128	46.942	7.639	10.542	1.00	43.41	C
ATOM	1150	N	LEU	A	129	48.907	6.715	12.425	1.00	18.32	N
ATOM	1151	CA	LEU	A	129	49.674	7.534	13.356	1.00	16.76	C
ATOM	1152	C	LEU	A	129	49.504	8.959	12.967	1.00	4.89	C
ATOM	1153	O	LEU	A	129	49.232	9.260	11.814	1.00	16.14	O
ATOM	1154	CB	LEU	A	129	51.205	7.191	13.261	1.00	17.91	C
ATOM	1155	CG	LEU	A	129	51.769	5.804	13.752	1.00	18.21	C
ATOM	1156	CD1	LEU	A	129	53.132	5.379	13.193	1.00	12.12	C
ATOM	1157	CD2	LEU	A	129	51.683	5.532	15.251	1.00	3.89	C
ATOM	1158	N	GLU	A	130	49.816	9.827	13.917	1.00	10.23	N
ATOM	1159	CA	GLU	A	130	49.912	11.268	13.691	1.00	13.22	C
ATOM	1160	C	GLU	A	130	51.128	11.544	12.775	1.00	23.44	C
ATOM	1161	O	GLU	A	130	52.249	11.162	13.090	1.00	21.23	O
ATOM	1162	CB	GLU	A	130	50.150	11.979	15.035	1.00	18.48	C
ATOM	1163	CG	GLU	A	130	50.754	13.376	14.886	1.00	77.44	C
ATOM	1164	CD	GLU	A	130	49.833	14.328	14.121	1.00	100.00	C
ATOM	1165	OE1	GLU	A	130	48.588	14.205	14.340	1.00	36.19	O
ATOM	1166	OE2	GLU	A	130	50.347	15.161	13.295	1.00	21.03	O
ATOM	1167	N	PRO	A	131	50.920	12.219	11.648	1.00	21.35	N
ATOM	1168	CA	PRO	A	131	52.023	12.409	10.731	1.00	14.78	C
ATOM	1169	C	PRO	A	131	53.201	13.132	11.265	1.00	14.98	C
ATOM	1170	O	PRO	A	131	54.325	12.847	10.853	1.00	20.99	O
ATOM	1171	CB	PRO	A	131	51.413	13.154	9.552	1.00	14.76	C
ATOM	1172	CG	PRO	A	131	50.071	13.485	9.949	1.00	20.99	C
ATOM	1173	CD	PRO	A	131	49.641	12.626	11.047	1.00	17.25	C
ATOM	1174	N	THR	A	132	52.986	14.095	12.159	1.00	18.77	N
ATOM	1175	CA	THR	A	132	54.131	14.838	12.689	1.00	16.44	C
ATOM	1176	C	THR	A	132	55.102	13.951	13.408	1.00	21.91	C
ATOM	1177	O	THR	A	132	56.317	14.088	13.234	1.00	24.17	O
ATOM	1178	CB	THR	A	132	53.716	15.907	13.606	1.00	23.45	C
ATOM	1179	OG1	THR	A	132	52.976	16.883	12.850	1.00	31.15	O

ATOM	1180	CG2	THR	A	132	54.969	16.519	14.341	1.00	9.28	C
ATOM	1181	N	ASN	A	133	54.551	12.970	14.122	1.00	28.59	N
ATOM	1182	CA	ASN	A	133	55.359	12.007	14.875	1.00	26.38	C
ATOM	1183	C	ASN	A	133	55.666	10.682	14.207	1.00	14.85	C
ATOM	1184	O	ASN	A	133	56.446	9.884	14.755	1.00	18.67	O
ATOM	1185	CB	ASN	A	133	54.661	11.699	16.168	1.00	23.70	C
ATOM	1186	CG	ASN	A	133	54.480	12.894	16.968	1.00	50.55	C
ATOM	1187	OD1	ASN	A	133	53.354	13.272	17.252	1.00	40.07	O
ATOM	1188	ND2	ASN	A	133	55.568	13.638	17.163	1.00	40.36	N
ATOM	1189	N	GLU	A	134	55.100	10.469	13.022	1.00	9.98	N
ATOM	1190	CA	GLU	A	134	55.237	9.210	12.365	1.00	9.66	C
ATOM	1191	C	GLU	A	134	56.648	8.530	12.274	1.00	13.86	C
ATOM	1192	O	GLU	A	134	56.814	7.388	12.706	1.00	22.89	O
ATOM	1193	CB	GLU	A	134	54.448	9.200	11.070	1.00	17.55	C
ATOM	1194	CG	GLU	A	134	54.750	7.930	10.227	1.00	20.89	C
ATOM	1195	CD	GLU	A	134	53.926	7.868	8.970	1.00	13.59	C
ATOM	1196	OE1	GLU	A	134	52.678	7.738	9.085	1.00	35.28	O
ATOM	1197	OE2	GLU	A	134	54.497	8.048	7.869	1.00	13.44	O
ATOM	1198	N	PRO	A	135	57.680	9.222	11.789	1.00	15.72	N
ATOM	1199	CA	PRO	A	135	59.014	8.600	11.699	1.00	18.91	C
ATOM	1200	C	PRO	A	135	59.544	8.174	13.073	1.00	18.68	C
ATOM	1201	O	PRO	A	135	60.072	7.069	13.271	1.00	15.69	O
ATOM	1202	CB	PRO	A	135	59.896	9.755	11.169	1.00	13.84	C
ATOM	1203	CG	PRO	A	135	59.036	10.514	10.350	1.00	9.78	C
ATOM	1204	CD	PRO	A	135	57.594	10.395	10.908	1.00	14.43	C
ATOM	1205	N	TYR	A	136	59.449	9.117	13.994	1.00	8.64	N
ATOM	1206	CA	TYR	A	136	59.873	8.915	15.324	1.00	13.27	C
ATOM	1207	C	TYR	A	136	59.056	7.728	15.907	1.00	16.84	C
ATOM	1208	O	TYR	A	136	59.578	6.903	16.658	1.00	12.90	O
ATOM	1209	CB	TYR	A	136	59.604	10.234	16.100	1.00	15.51	C
ATOM	1210	CG	TYR	A	136	59.912	10.168	17.614	1.00	18.26	C
ATOM	1211	CD1	TYR	A	136	61.200	10.062	18.072	1.00	20.53	C
ATOM	1212	CD2	TYR	A	136	58.904	10.150	18.568	1.00	17.38	C
ATOM	1213	CE1	TYR	A	136	61.484	9.959	19.440	1.00	30.44	C
ATOM	1214	CE2	TYR	A	136	59.184	10.084	19.953	1.00	9.85	C
ATOM	1215	CZ	TYR	A	136	60.476	9.949	20.377	1.00	20.65	C
ATOM	1216	OH	TYR	A	136	60.792	9.873	21.734	1.00	24.41	O
ATOM	1217	N	ALA	A	137	57.760	7.687	15.638	1.00	7.19	N
ATOM	1218	CA	ALA	A	137	56.923	6.633	16.227	1.00	12.68	C
ATOM	1219	C	ALA	A	137	57.345	5.265	15.737	1.00	15.21	C
ATOM	1220	O	ALA	A	137	57.425	4.272	16.488	1.00	14.58	O
ATOM	1221	CB	ALA	A	137	55.517	6.849	15.871	1.00	11.40	C
ATOM	1222	N	ILE	A	138	57.567	5.213	14.447	1.00	8.93	N
ATOM	1223	CA	ILE	A	138	57.954	3.971	13.831	1.00	11.77	C
ATOM	1224	C	ILE	A	138	59.246	3.494	14.492	1.00	16.20	C

ATOM	1225	O	ILE	A	138	59.307	2.377	14.970	1.00	13.79	O
ATOM	1226	CB	ILE	A	138	58.064	4.172	12.316	1.00	17.85	C
ATOM	1227	CG1	ILE	A	138	56.680	4.473	11.757	1.00	28.21	C
ATOM	1228	CG2	ILE	A	138	58.674	2.986	11.602	1.00	9.81	C
ATOM	1229	CD1	ILE	A	138	55.695	3.376	11.970	1.00	18.17	C
ATOM	1230	N	ALA	A	139	60.243	4.361	14.625	1.00	11.54	N
ATOM	1231	CA	ALA	A	139	61.494	3.937	15.288	1.00	13.22	C
ATOM	1232	C	ALA	A	139	61.256	3.364	16.675	1.00	18.73	C
ATOM	1233	O	ALA	A	139	61.791	2.318	17.031	1.00	20.44	O
ATOM	1234	CB	ALA	A	139	62.434	5.073	15.390	1.00	13.62	C
ATOM	1235	N	LYS	A	140	60.397	4.033	17.448	1.00	16.36	N
ATOM	1236	CA	LYS	A	140	60.083	3.600	18.815	1.00	15.14	C
ATOM	1237	C	LYS	A	140	59.392	2.262	18.824	1.00	15.18	C
ATOM	1238	O	LYS	A	140	59.824	1.346	19.475	1.00	21.42	O
ATOM	1239	CB	LYS	A	140	59.193	4.606	19.525	1.00	17.86	C
ATOM	1240	CG	LYS	A	140	59.925	5.806	20.152	1.00	21.11	C
ATOM	1241	CD	LYS	A	140	61.208	5.478	20.958	1.00	16.75	C
ATOM	1242	CE	LYS	A	140	61.664	6.735	21.835	1.00	10.06	C
ATOM	1243	NZ	LYS	A	140	62.688	6.496	22.921	1.00	14.40	N
ATOM	1244	N	ILE	A	141	58.356	2.116	18.027	1.00	11.49	N
ATOM	1245	CA	ILE	A	141	57.703	0.828	17.977	1.00	17.92	C
ATOM	1246	C	ILE	A	141	58.729	-0.282	17.577	1.00	13.46	C
ATOM	1247	O	ILE	A	141	58.730	-1.374	18.148	1.00	13.92	O
ATOM	1248	CB	ILE	A	141	56.497	0.925	17.019	1.00	22.59	C
ATOM	1249	CG1	ILE	A	141	55.466	1.906	17.557	1.00	17.61	C
ATOM	1250	CG2	ILE	A	141	55.863	-0.411	16.700	1.00	10.49	C
ATOM	1251	CD1	ILE	A	141	54.530	2.327	16.449	1.00	13.43	C
ATOM	1252	N	ALA	A	142	59.637	0.028	16.650	1.00	10.29	N
ATOM	1253	CA	ALA	A	142	60.657	-0.931	16.228	1.00	7.15	C
ATOM	1254	C	ALA	A	142	61.456	-1.301	17.456	1.00	16.58	C
ATOM	1255	O	ALA	A	142	61.839	-2.454	17.621	1.00	13.04	O
ATOM	1256	CB	ALA	A	142	61.604	-0.288	15.130	1.00	4.44	C
ATOM	1257	N	GLY	A	143	61.703	-0.307	18.316	1.00	9.56	N
ATOM	1258	CA	GLY	A	143	62.448	-0.525	19.527	1.00	5.15	C
ATOM	1259	C	GLY	A	143	61.770	-1.555	20.430	1.00	16.36	C
ATOM	1260	O	GLY	A	143	62.392	-2.482	20.967	1.00	14.11	O
ATOM	1261	N	ILE	A	144	60.476	-1.418	20.564	1.00	20.33	N
ATOM	1262	CA	ILE	A	144	59.725	-2.314	21.407	1.00	15.35	C
ATOM	1263	C	ILE	A	144	59.706	-3.732	20.859	1.00	19.84	C
ATOM	1264	O	ILE	A	144	59.836	-4.700	21.608	1.00	17.93	O
ATOM	1265	CB	ILE	A	144	58.317	-1.819	21.559	1.00	10.60	C
ATOM	1266	CG1	ILE	A	144	58.311	-0.610	22.516	1.00	9.80	C
ATOM	1267	CG2	ILE	A	144	57.410	-2.928	22.122	1.00	9.60	C
ATOM	1268	CD1	ILE	A	144	57.022	0.076	22.517	1.00	18.32	C
ATOM	1269	N	LYS	A	145	59.520	-3.841	19.556	1.00	7.20	N

ATOM	1270	CA	LYS	A	145	59.459	-5.139	18.926	1.00	7.64	C
ATOM	1271	C	LYS	A	145	60.840	-5.788	18.931	1.00	15.32	C
ATOM	1272	O	LYS	A	145	60.923	-6.989	18.981	1.00	14.76	O
ATOM	1273	CB	LYS	A	145	58.891	-5.001	17.516	1.00	11.25	C
ATOM	1274	CG	LYS	A	145	57.414	-4.581	17.489	1.00	12.13	C
ATOM	1275	CD	LYS	A	145	56.642	-5.434	18.495	1.00	25.23	C
ATOM	1276	CE	LYS	A	145	55.189	-4.995	18.692	1.00	13.64	C
ATOM	1277	NZ	LYS	A	145	54.441	-6.111	19.392	1.00	11.94	N
ATOM	1278	N	LEU	A	146	61.934	-5.011	18.986	1.00	26.98	N
ATOM	1279	CA	LEU	A	146	63.261	-5.642	19.167	1.00	19.72	C
ATOM	1280	C	LEU	A	146	63.262	-6.316	20.542	1.00	18.20	C
ATOM	1281	O	LEU	A	146	63.590	-7.511	20.703	1.00	19.86	O
ATOM	1282	CB	LEU	A	146	64.398	-4.618	19.150	1.00	13.56	C
ATOM	1283	CG	LEU	A	146	64.895	-4.258	17.759	1.00	21.84	C
ATOM	1284	CD1	LEU	A	146	65.672	-2.945	17.817	1.00	17.94	C
ATOM	1285	CD2	LEU	A	146	65.745	-5.397	17.102	1.00	16.10	C
ATOM	1286	N	CYS	A	147	62.931	-5.523	21.548	1.00	7.91	N
ATOM	1287	CA	CYS	A	147	62.875	-6.064	22.893	1.00	9.14	C
ATOM	1288	C	CYS	A	147	62.072	-7.378	22.945	1.00	22.72	C
ATOM	1289	O	CYS	A	147	62.568	-8.401	23.383	1.00	16.90	O
ATOM	1290	CB	CYS	A	147	62.232	-5.058	23.809	1.00	12.63	C
ATOM	1291	SG	CYS	A	147	63.411	-3.823	24.316	1.00	15.02	S
ATOM	1292	N	GLU	A	148	60.823	-7.352	22.508	1.00	20.03	N
ATOM	1293	CA	GLU	A	148	60.016	-8.555	22.567	1.00	16.09	C
ATOM	1294	C	GLU	A	148	60.685	-9.715	21.802	1.00	22.61	C
ATOM	1295	O	GLU	A	148	60.651	-10.888	22.226	1.00	12.05	O
ATOM	1296	CB	GLU	A	148	58.597	-8.268	22.046	1.00	14.66	C
ATOM	1297	CG	GLU	A	148	57.864	-7.189	22.840	1.00	11.45	C
ATOM	1298	CD	GLU	A	148	56.471	-6.821	22.277	1.00	11.75	C
ATOM	1299	OE1	GLU	A	148	56.117	-7.055	21.080	1.00	11.65	O
ATOM	1300	OE2	GLU	A	148	55.728	-6.231	23.081	1.00	22.56	O
ATOM	1301	N	SER	A	149	61.368	-9.377	20.715	1.00	15.57	N
ATOM	1302	CA	SER	A	149	61.938	-10.428	19.887	1.00	10.21	C
ATOM	1303	C	SER	A	149	63.040	-11.245	20.502	1.00	15.83	C
ATOM	1304	O	SER	A	149	63.102	-12.458	20.291	1.00	12.72	O
ATOM	1305	CB	SER	A	149	62.270	-9.936	18.488	1.00	9.44	C
ATOM	1306	OG	SER	A	149	61.053	-9.650	17.782	1.00	15.91	O
ATOM	1307	N	TYR	A	150	63.910	-10.546	21.224	1.00	18.44	N
ATOM	1308	CA	TYR	A	150	65.065	-11.100	21.948	1.00	20.50	C
ATOM	1309	C	TYR	A	150	64.514	-11.848	23.158	1.00	21.87	C
ATOM	1310	O	TYR	A	150	64.939	-12.949	23.486	1.00	31.39	O
ATOM	1311	CB	TYR	A	150	66.005	-9.950	22.425	1.00	13.71	C
ATOM	1312	CG	TYR	A	150	66.994	-9.509	21.365	1.00	14.13	C
ATOM	1313	CD1	TYR	A	150	66.611	-8.673	20.317	1.00	14.64	C
ATOM	1314	CD2	TYR	A	150	68.288	-10.000	21.360	1.00	18.32	C

ATOM	1315	CE1	TYR	A	150	67.487	-8.390	19.278	1.00	11.91	C
ATOM	1316	CE2	TYR	A	150	69.198	-9.682	20.345	1.00	11.10	C
ATOM	1317	CZ	TYR	A	150	68.804	-8.900	19.326	1.00	20.95	C
ATOM	1318	OH	TYR	A	150	69.739	-8.685	18.333	1.00	27.73	O
ATOM	1319	N	ASN	A	151	63.536	-11.249	23.801	1.00	14.83	N
ATOM	1320	CA	ASN	A	151	62.903	-11.889	24.937	1.00	23.62	C
ATOM	1321	C	ASN	A	151	62.417	-13.244	24.410	1.00	28.53	C
ATOM	1322	O	ASN	A	151	62.630	-14.248	25.072	1.00	25.89	O
ATOM	1323	CB	ASN	A	151	61.655	-11.113	25.439	1.00	20.95	C
ATOM	1324	CG	ASN	A	151	61.988	-9.867	26.284	1.00	15.07	C
ATOM	1325	OD1	ASN	A	151	61.126	-9.020	26.466	1.00	26.72	O
ATOM	1326	ND2	ASN	A	151	63.231	-9.709	26.700	1.00	6.31	N
ATOM	1327	N	ARG	A	152	61.731	-13.249	23.259	1.00	19.91	N
ATOM	1328	CA	ARG	A	152	61.129	-14.465	22.687	1.00	17.62	C
ATOM	1329	C	ARG	A	152	62.090	-15.523	22.188	1.00	21.34	C
ATOM	1330	O	ARG	A	152	61.959	-16.687	22.542	1.00	15.44	O
ATOM	1331	CB	ARG	A	152	60.086	-14.148	21.610	1.00	15.30	C
ATOM	1332	CG	ARG	A	152	58.672	-13.754	22.157	1.00	17.22	C
ATOM	1333	CD	ARG	A	152	57.652	-13.297	21.049	1.00	9.11	C
ATOM	1334	NE	ARG	A	152	57.161	-14.419	20.241	1.00	21.05	N
ATOM	1335	CZ	ARG	A	152	57.159	-14.447	18.912	1.00	28.61	C
ATOM	1336	NH1	ARG	A	152	57.590	-13.387	18.221	1.00	21.98	N
ATOM	1337	NH2	ARG	A	152	56.717	-15.528	18.262	1.00	26.11	N
ATOM	1338	N	GLN	A	153	63.098	-15.104	21.434	1.00	16.54	N
ATOM	1339	CA	GLN	A	153	64.044	-16.036	20.842	1.00	9.74	C
ATOM	1340	C	GLN	A	153	65.082	-16.443	21.807	1.00	16.70	C
ATOM	1341	O	GLN	A	153	65.529	-17.545	21.763	1.00	24.35	O
ATOM	1342	CB	GLN	A	153	64.789	-15.372	19.714	1.00	8.99	C
ATOM	1343	CG	GLN	A	153	65.935	-16.225	19.116	1.00	4.63	C
ATOM	1344	CD	GLN	A	153	66.315	-15.637	17.762	1.00	14.17	C
ATOM	1345	OE1	GLN	A	153	65.611	-14.763	17.254	1.00	12.53	O
ATOM	1346	NE2	GLN	A	153	67.466	-16.024	17.228	1.00	13.38	N
ATOM	1347	N	TYR	A	154	65.566	-15.518	22.608	1.00	14.35	N
ATOM	1348	CA	TYR	A	154	66.677	-15.839	23.483	1.00	12.16	C
ATOM	1349	C	TYR	A	154	66.323	-15.930	24.954	1.00	19.06	C
ATOM	1350	O	TYR	A	154	67.185	-16.207	25.777	1.00	25.59	O
ATOM	1351	CB	TYR	A	154	67.829	-14.816	23.326	1.00	16.89	C
ATOM	1352	CG	TYR	A	154	68.418	-14.733	21.943	1.00	17.53	C
ATOM	1353	CD1	TYR	A	154	69.259	-15.726	21.467	1.00	18.91	C
ATOM	1354	CD2	TYR	A	154	68.080	-13.712	21.091	1.00	13.97	C
ATOM	1355	CE1	TYR	A	154	69.782	-15.686	20.190	1.00	10.98	C
ATOM	1356	CE2	TYR	A	154	68.621	-13.639	19.806	1.00	23.81	C
ATOM	1357	CZ	TYR	A	154	69.488	-14.634	19.380	1.00	23.08	C
ATOM	1358	OH	TYR	A	154	70.002	-14.619	18.118	1.00	23.87	O
ATOM	1359	N	GLY	A	155	65.080	-15.686	25.313	1.00	12.08	N

ATOM	1360	CA	GLY	A	155	64.747	-15.702	26.731	1.00	15.80	C
ATOM	1361	C	GLY	A	155	65.323	-14.498	27.580	1.00	33.97	C
ATOM	1362	O	GLY	A	155	65.491	-14.640	28.789	1.00	25.76	O
ATOM	1363	N	ARG	A	156	65.564	-13.318	26.981	1.00	25.91	N
ATOM	1364	CA	ARG	A	156	66.066	-12.146	27.734	1.00	14.13	C
ATOM	1365	C	ARG	A	156	64.971	-11.486	28.581	1.00	16.23	C
ATOM	1366	O	ARG	A	156	63.802	-11.919	28.583	1.00	22.61	O
ATOM	1367	CB	ARG	A	156	66.601	-11.124	26.750	1.00	13.16	C
ATOM	1368	CG	ARG	A	156	67.875	-11.570	26.099	1.00	15.18	C
ATOM	1369	CD	ARG	A	156	68.930	-11.418	27.121	1.00	26.42	C
ATOM	1370	NE	ARG	A	156	70.200	-11.912	26.633	1.00	21.25	N
ATOM	1371	CZ	ARG	A	156	71.092	-12.555	27.386	1.00	42.25	C
ATOM	1372	NH1	ARG	A	156	70.870	-12.795	28.679	1.00	20.02	N
ATOM	1373	NH2	ARG	A	156	72.221	-12.966	26.843	1.00	20.88	N
ATOM	1374	N	ASP	A	157	65.343	-10.446	29.321	1.00	16.00	N
ATOM	1375	CA	ASP	A	157	64.370	-9.749	30.166	1.00	16.20	C
ATOM	1376	C	ASP	A	157	64.444	-8.245	29.841	1.00	19.20	C
ATOM	1377	O	ASP	A	157	64.865	-7.429	30.650	1.00	10.71	O
ATOM	1378	CB	ASP	A	157	64.609	-10.061	31.652	1.00	16.50	C
ATOM	1379	CG	ASP	A	157	63.489	-9.560	32.566	1.00	26.45	C
ATOM	1380	OD1	ASP	A	157	62.433	-9.060	32.108	1.00	26.82	O
ATOM	1381	OD2	ASP	A	157	63.673	-9.653	33.784	1.00	21.88	O
ATOM	1382	N	TYR	A	158	64.038	-7.921	28.620	1.00	19.41	N
ATOM	1383	CA	TYR	A	158	64.099	-6.564	28.083	1.00	18.96	C
ATOM	1384	C	TYR	A	158	62.688	-5.977	28.127	1.00	22.62	C
ATOM	1385	O	TYR	A	158	61.854	-6.296	27.282	1.00	10.12	O
ATOM	1386	CB	TYR	A	158	64.562	-6.661	26.631	1.00	16.34	C
ATOM	1387	CG	TYR	A	158	65.982	-7.166	26.484	1.00	12.04	C
ATOM	1388	CD1	TYR	A	158	66.789	-7.415	27.621	1.00	13.76	C
ATOM	1389	CD2	TYR	A	158	66.544	-7.349	25.218	1.00	16.35	C
ATOM	1390	CE1	TYR	A	158	68.135	-7.786	27.482	1.00	8.18	C
ATOM	1391	CE2	TYR	A	158	67.886	-7.732	25.060	1.00	13.73	C
ATOM	1392	CZ	TYR	A	158	68.676	-7.942	26.186	1.00	24.45	C
ATOM	1393	OH	TYR	A	158	69.993	-8.338	25.997	1.00	14.36	O
ATOM	1394	N	ARG	A	159	62.423	-5.200	29.175	1.00	23.53	N
ATOM	1395	CA	ARG	A	159	61.105	-4.603	29.483	1.00	21.15	C
ATOM	1396	C	ARG	A	159	60.930	-3.172	28.878	1.00	23.55	C
ATOM	1397	O	ARG	A	159	61.911	-2.566	28.424	1.00	18.12	O
ATOM	1398	CB	ARG	A	159	60.891	-4.608	31.034	1.00	21.68	C
ATOM	1399	CG	ARG	A	159	60.986	-6.029	31.722	1.00	16.41	C
ATOM	1400	CD	ARG	A	159	61.135	-6.052	33.233	1.00	18.10	C
ATOM	1401	NE	ARG	A	159	61.305	-7.402	33.772	1.00	19.25	N
ATOM	1402	CZ	ARG	A	159	61.164	-7.720	35.058	1.00	36.67	C
ATOM	1403	NH1	ARG	A	159	60.886	-6.776	35.962	1.00	15.32	N
ATOM	1404	NH2	ARG	A	159	61.309	-8.986	35.448	1.00	11.79	N

ATOM	1405	N	SER	A	160	59.689	-2.661	28.859	1.00	24.44	N
ATOM	1406	CA	SER	A	160	59.312	-1.393	28.200	1.00	21.59	C
ATOM	1407	C	SER	A	160	58.242	-0.577	28.950	1.00	25.07	C
ATOM	1408	O	SER	A	160	57.257	-1.127	29.454	1.00	17.02	O
ATOM	1409	CB	SER	A	160	58.719	-1.747	26.797	1.00	13.05	C
ATOM	1410	OG	SER	A	160	59.782	-1.897	25.885	1.00	37.57	O
ATOM	1411	N	VAL	A	161	58.378	0.742	28.927	1.00	21.01	N
ATOM	1412	CA	VAL	A	161	57.369	1.644	29.509	1.00	9.70	C
ATOM	1413	C	VAL	A	161	57.068	2.747	28.504	1.00	16.77	C
ATOM	1414	O	VAL	A	161	57.955	3.149	27.729	1.00	16.33	O
ATOM	1415	CB	VAL	A	161	57.806	2.248	30.862	1.00	17.94	C
ATOM	1416	CG1	VAL	A	161	57.873	1.185	31.984	1.00	16.16	C
ATOM	1417	CG2	VAL	A	161	59.137	2.992	30.750	1.00	21.10	C
ATOM	1418	N	MET	A	162	55.794	3.147	28.443	1.00	22.46	N
ATOM	1419	CA	MET	A	162	55.296	4.185	27.513	1.00	19.23	C
ATOM	1420	C	MET	A	162	54.880	5.312	28.397	1.00	25.19	C
ATOM	1421	O	MET	A	162	53.788	5.269	28.961	1.00	18.35	O
ATOM	1422	CB	MET	A	162	53.979	3.796	26.850	1.00	15.55	C
ATOM	1423	CG	MET	A	162	54.013	2.630	25.949	1.00	37.79	C
ATOM	1424	SD	MET	A	162	54.354	3.100	24.235	1.00	52.07	S
ATOM	1425	CE	MET	A	162	56.193	3.134	24.410	1.00	36.30	C
ATOM	1426	N	PRO	A	163	55.730	6.313	28.521	1.00	18.43	N
ATOM	1427	CA	PRO	A	163	55.390	7.472	29.337	1.00	17.76	C
ATOM	1428	C	PRO	A	163	54.300	8.384	28.667	1.00	21.23	C
ATOM	1429	O	PRO	A	163	54.208	8.448	27.433	1.00	15.20	O
ATOM	1430	CB	PRO	A	163	56.727	8.196	29.423	1.00	11.43	C
ATOM	1431	CG	PRO	A	163	57.352	7.874	28.031	1.00	13.99	C
ATOM	1432	CD	PRO	A	163	57.086	6.401	27.949	1.00	12.24	C
ATOM	1433	N	THR	A	164	53.478	9.060	29.478	1.00	13.95	N
ATOM	1434	CA	THR	A	164	52.581	10.121	28.963	1.00	25.82	C
ATOM	1435	C	THR	A	164	53.406	11.441	28.781	1.00	19.67	C
ATOM	1436	O	THR	A	164	54.633	11.393	28.868	1.00	13.97	O
ATOM	1437	CB	THR	A	164	51.373	10.391	29.903	1.00	25.51	C
ATOM	1438	OG1	THR	A	164	50.470	11.321	29.267	1.00	14.77	O
ATOM	1439	CG2	THR	A	164	51.818	10.886	31.298	1.00	9.06	C
ATOM	1440	N	ASN	A	165	52.751	12.589	28.556	1.00	14.99	N
ATOM	1441	CA	ASN	A	165	53.448	13.901	28.481	1.00	7.83	C
ATOM	1442	C	ASN	A	165	54.167	14.064	29.824	1.00	11.21	C
ATOM	1443	O	ASN	A	165	53.554	13.929	30.894	1.00	17.66	O
ATOM	1444	CB	ASN	A	165	52.434	15.061	28.416	1.00	14.48	C
ATOM	1445	CG	ASN	A	165	51.492	14.941	27.262	1.00	23.70	C
ATOM	1446	OD1	ASN	A	165	51.939	14.800	26.129	1.00	22.37	O
ATOM	1447	ND2	ASN	A	165	50.173	14.925	27.539	1.00	27.22	N
ATOM	1448	N	LEU	A	166	55.418	14.490	29.777	1.00	8.23	N
ATOM	1449	CA	LEU	A	166	56.187	14.604	30.994	1.00	14.40	C

ATOM	1450	C	LEU	A	166	56.629	16.017	31.120	1.00	25.05	C
ATOM	1451	O	LEU	A	166	56.624	16.718	30.125	1.00	25.09	O
ATOM	1452	CB	LEU	A	166	57.460	13.743	30.870	1.00	17.48	C
ATOM	1453	CG	LEU	A	166	57.423	12.218	30.652	1.00	16.63	C
ATOM	1454	CD1	LEU	A	166	58.837	11.639	31.000	1.00	22.52	C
ATOM	1455	CD2	LEU	A	166	56.336	11.539	31.514	1.00	7.46	C
ATOM	1456	N	TYR	A	167	57.146	16.391	32.300	1.00	19.78	N
ATOM	1457	CA	TYR	A	167	57.678	17.760	32.511	1.00	18.58	C
ATOM	1458	C	TYR	A	167	58.534	17.763	33.767	1.00	15.53	C
ATOM	1459	O	TYR	A	167	58.474	16.852	34.575	1.00	16.71	O
ATOM	1460	CB	TYR	A	167	56.509	18.778	32.665	1.00	18.33	C
ATOM	1461	CG	TYR	A	167	55.671	18.561	33.931	1.00	14.23	C
ATOM	1462	CD1	TYR	A	167	54.624	17.618	33.977	1.00	13.35	C
ATOM	1463	CD2	TYR	A	167	55.984	19.258	35.106	1.00	16.52	C
ATOM	1464	CE1	TYR	A	167	53.889	17.446	35.146	1.00	21.17	C
ATOM	1465	CE2	TYR	A	167	55.302	19.084	36.264	1.00	8.26	C
ATOM	1466	CZ	TYR	A	167	54.228	18.203	36.296	1.00	23.56	C
ATOM	1467	OH	TYR	A	167	53.526	18.078	37.504	1.00	22.81	O
ATOM	1468	N	GLY	A	168	59.334	18.797	33.952	1.00	16.59	N
ATOM	1469	CA	GLY	A	168	60.158	18.817	35.152	1.00	18.21	C
ATOM	1470	C	GLY	A	168	61.534	19.428	34.880	1.00	13.69	C
ATOM	1471	O	GLY	A	168	61.746	20.028	33.837	1.00	16.52	O
ATOM	1472	N	PRO	A	169	62.473	19.263	35.817	1.00	20.33	N
ATOM	1473	CA	PRO	A	169	63.801	19.822	35.656	1.00	16.07	C
ATOM	1474	C	PRO	A	169	64.430	19.353	34.387	1.00	27.18	C
ATOM	1475	O	PRO	A	169	64.305	18.186	33.981	1.00	21.23	O
ATOM	1476	CB	PRO	A	169	64.595	19.206	36.805	1.00	17.28	C
ATOM	1477	CG	PRO	A	169	63.649	18.919	37.830	1.00	19.89	C
ATOM	1478	CD	PRO	A	169	62.263	18.772	37.189	1.00	22.47	C
ATOM	1479	N	HIS	A	170	65.226	20.235	33.829	1.00	19.48	N
ATOM	1480	CA	HIS	A	170	65.952	19.877	32.638	1.00	25.56	C
ATOM	1481	C	HIS	A	170	65.096	19.707	31.428	1.00	29.15	C
ATOM	1482	O	HIS	A	170	65.553	19.091	30.479	1.00	29.71	O
ATOM	1483	CB	HIS	A	170	66.783	18.600	32.845	1.00	28.94	C
ATOM	1484	CG	HIS	A	170	67.703	18.671	34.034	1.00	33.88	C
ATOM	1485	ND1	HIS	A	170	68.975	19.203	33.969	1.00	25.46	N
ATOM	1486	CD2	HIS	A	170	67.518	18.298	35.326	1.00	34.77	C
ATOM	1487	CE1	HIS	A	170	69.531	19.151	35.166	1.00	25.63	C
ATOM	1488	NE2	HIS	A	170	68.673	18.603	36.008	1.00	31.72	N
ATOM	1489	N	ASP	A	171	63.881	20.245	31.440	1.00	21.52	N
ATOM	1490	CA	ASP	A	171	63.041	20.267	30.218	1.00	28.63	C
ATOM	1491	C	ASP	A	171	63.630	21.459	29.359	1.00	41.94	C
ATOM	1492	O	ASP	A	171	64.534	22.171	29.835	1.00	29.69	O
ATOM	1493	CB	ASP	A	171	61.552	20.558	30.602	1.00	26.40	C
ATOM	1494	CG	ASP	A	171	60.552	20.097	29.540	1.00	22.32	C

ATOM	1495	OD1	ASP	A	171	60.890	20.067	28.325	1.00	32.03	O
ATOM	1496	OD2	ASP	A	171	59.427	19.719	29.916	1.00	42.13	O
ATOM	1497	N	ASN	A	172	63.141	21.712	28.137	1.00	42.08	N
ATOM	1498	CA	ASN	A	172	63.616	22.893	27.388	1.00	35.95	C
ATOM	1499	C	ASN	A	172	62.665	24.056	27.674	1.00	33.71	C
ATOM	1500	O	ASN	A	172	61.586	24.102	27.104	1.00	32.69	O
ATOM	1501	CB	ASN	A	172	63.632	22.667	25.869	1.00	41.60	C
ATOM	1502	CG	ASN	A	172	63.807	23.987	25.086	1.00	39.09	C
ATOM	1503	OD1	ASN	A	172	62.973	24.347	24.259	1.00	83.94	O
ATOM	1504	ND2	ASN	A	172	64.855	24.740	25.418	1.00	65.07	N
ATOM	1505	N	PHE	A	173	63.021	24.953	28.583	1.00	31.93	N
ATOM	1506	CA	PHE	A	173	62.082	26.030	28.944	1.00	48.24	C
ATOM	1507	C	PHE	A	173	61.989	27.260	28.045	1.00	69.01	C
ATOM	1508	O	PHE	A	173	62.278	28.395	28.465	1.00	58.79	O
ATOM	1509	CB	PHE	A	173	62.225	26.459	30.390	1.00	43.43	C
ATOM	1510	CG	PHE	A	173	61.867	25.399	31.356	1.00	34.19	C
ATOM	1511	CD1	PHE	A	173	62.810	24.488	31.751	1.00	24.68	C
ATOM	1512	CD2	PHE	A	173	60.621	25.354	31.925	1.00	24.84	C
ATOM	1513	CE1	PHE	A	173	62.524	23.548	32.682	1.00	23.64	C
ATOM	1514	CE2	PHE	A	173	60.305	24.366	32.804	1.00	31.32	C
ATOM	1515	CZ	PHE	A	173	61.263	23.457	33.192	1.00	24.30	C
ATOM	1516	N	HIS	A	174	61.510	27.036	26.831	1.00	68.16	N
ATOM	1517	CA	HIS	A	174	61.401	28.109	25.871	1.00	64.53	C
ATOM	1518	C	HIS	A	174	59.973	28.221	25.400	1.00	71.58	C
ATOM	1519	O	HIS	A	174	59.309	27.186	25.249	1.00	73.20	O
ATOM	1520	CB	HIS	A	174	62.418	27.870	24.736	1.00	71.71	C
ATOM	1521	CG	HIS	A	174	63.835	27.868	25.229	1.00	92.29	C
ATOM	1522	ND1	HIS	A	174	64.921	27.539	24.440	1.00100.00		N
ATOM	1523	CD2	HIS	A	174	64.338	28.133	26.463	1.00100.00		C
ATOM	1524	CE1	HIS	A	174	66.032	27.628	25.160	1.00100.00		C
ATOM	1525	NE2	HIS	A	174	65.705	27.981	26.393	1.00100.00		N
ATOM	1526	N	PRO	A	175	59.469	29.461	25.262	1.00	65.71	N
ATOM	1527	CA	PRO	A	175	58.109	29.658	24.770	1.00	55.72	C
ATOM	1528	C	PRO	A	175	58.233	29.297	23.267	1.00	75.83	C
ATOM	1529	O	PRO	A	175	57.224	29.226	22.554	1.00	69.59	O
ATOM	1530	CB	PRO	A	175	57.866	31.142	25.026	1.00	49.14	C
ATOM	1531	CG	PRO	A	175	59.258	31.790	24.901	1.00	42.23	C
ATOM	1532	CD	PRO	A	175	60.286	30.695	25.109	1.00	49.59	C
ATOM	1533	N	SER	A	176	59.480	28.954	22.879	1.00	85.09	N
ATOM	1534	CA	SER	A	176	59.954	28.474	21.548	1.00	81.18	C
ATOM	1535	C	SER	A	176	59.660	26.965	21.343	1.00	73.90	C
ATOM	1536	O	SER	A	176	59.617	26.458	20.213	1.00	57.03	O
ATOM	1537	CB	SER	A	176	61.493	28.666	21.447	1.00	71.32	C
ATOM	1538	OG	SER	A	176	62.048	29.349	22.578	1.00	51.93	O
ATOM	1539	N	ASN	A	177	59.520	26.276	22.480	1.00	66.23	N

ATOM	1540	CA	ASN	A	177	59.274	24.847	22.619	1.00 56.41	C
ATOM	1541	C	ASN	A	177	57.810	24.497	22.353	1.00 60.91	C
ATOM	1542	O	ASN	A	177	56.914	25.215	22.811	1.00 55.58	O
ATOM	1543	CB	ASN	A	177	59.619	24.469	24.065	1.00 50.45	C
ATOM	1544	CG	ASN	A	177	59.562	22.970	24.319	1.00 66.57	C
ATOM	1545	OD1	ASN	A	177	59.095	22.216	23.476	1.00100.00	O
ATOM	1546	ND2	ASN	A	177	60.099	22.546	25.464	1.00 35.61	N
ATOM	1547	N	SER	A	178	57.583	23.387	21.627	1.00 57.10	N
ATOM	1548	CA	SER	A	178	56.234	22.853	21.279	1.00 50.50	C
ATOM	1549	C	SER	A	178	55.557	22.159	22.491	1.00 76.24	C
ATOM	1550	O	SER	A	178	54.575	21.400	22.304	1.00 99.63	O
ATOM	1551	CB	SER	A	178	56.316	21.800	20.118	1.00 10.17	C
ATOM	1552	OG	SER	A	178	57.397	22.112	19.217	1.00 71.69	O
ATOM	1553	N	HIS	A	179	56.134	22.284	23.694	1.00 37.39	N
ATOM	1554	CA	HIS	A	179	55.569	21.587	24.855	1.00 30.96	C
ATOM	1555	C	HIS	A	179	54.961	22.616	25.767	1.00 21.93	C
ATOM	1556	O	HIS	A	179	55.641	23.598	26.138	1.00 25.17	O
ATOM	1557	CB	HIS	A	179	56.634	20.683	25.575	1.00 36.20	C
ATOM	1558	CG	HIS	A	179	56.973	19.419	24.835	1.00 42.90	C
ATOM	1559	ND1	HIS	A	179	56.973	19.335	23.457	1.00 49.52	N
ATOM	1560	CD2	HIS	A	179	57.323	18.190	25.278	1.00 52.42	C
ATOM	1561	CE1	HIS	A	179	57.283	18.109	23.084	1.00 44.78	C
ATOM	1562	NE2	HIS	A	179	57.500	17.393	24.168	1.00 50.49	N
ATOM	1563	N	VAL	A	180	53.661	22.454	26.038	1.00 19.14	N
ATOM	1564	CA	VAL	A	180	52.886	23.449	26.789	1.00 29.03	C
ATOM	1565	C	VAL	A	180	53.373	23.890	28.142	1.00 31.29	C
ATOM	1566	O	VAL	A	180	53.348	25.075	28.447	1.00 19.55	O
ATOM	1567	CB	VAL	A	180	51.403	23.115	26.914	1.00 35.47	C
ATOM	1568	CG1	VAL	A	180	50.630	24.399	27.217	1.00 35.84	C
ATOM	1569	CG2	VAL	A	180	50.923	22.550	25.663	1.00 36.11	C
ATOM	1570	N	ILE	A	181	53.684	22.935	29.005	1.00 26.57	N
ATOM	1571	CA	ILE	A	181	54.138	23.285	30.360	1.00 24.49	C
ATOM	1572	C	ILE	A	181	55.371	24.213	30.361	1.00 16.51	C
ATOM	1573	O	ILE	A	181	55.326	25.315	30.909	1.00 24.42	O
ATOM	1574	CB	ILE	A	181	54.285	22.018	31.264	1.00 20.20	C
ATOM	1575	CG1	ILE	A	181	52.878	21.428	31.528	1.00 18.22	C
ATOM	1576	CG2	ILE	A	181	55.014	22.315	32.581	1.00 13.37	C
ATOM	1577	CD1	ILE	A	181	52.867	20.086	32.286	1.00 8.03	C
ATOM	1578	N	PRO	A	182	56.452	23.779	29.718	1.00 22.21	N
ATOM	1579	CA	PRO	A	182	57.664	24.605	29.640	1.00 22.07	C
ATOM	1580	C	PRO	A	182	57.379	25.852	28.828	1.00 24.18	C
ATOM	1581	O	PRO	A	182	57.811	26.949	29.210	1.00 18.35	O
ATOM	1582	CB	PRO	A	182	58.682	23.725	28.890	1.00 24.97	C
ATOM	1583	CG	PRO	A	182	57.925	22.473	28.471	1.00 25.77	C
ATOM	1584	CD	PRO	A	182	56.727	22.359	29.401	1.00 18.23	C

ATOM	1585	N	ALA	A	183	56.628	25.707	27.729	1.00	21.45	N
ATOM	1586	CA	ALA	A	183	56.261	26.896	26.943	1.00	21.66	C
ATOM	1587	C	ALA	A	183	55.464	27.900	27.811	1.00	26.10	C
ATOM	1588	O	ALA	A	183	55.773	29.091	27.856	1.00	19.50	O
ATOM	1589	CB	ALA	A	183	55.473	26.513	25.703	1.00	13.26	C
ATOM	1590	N	LEU	A	184	54.472	27.389	28.543	1.00	23.34	N
ATOM	1591	CA	LEU	A	184	53.642	28.215	29.401	1.00	19.05	C
ATOM	1592	C	LEU	A	184	54.312	28.693	30.655	1.00	21.91	C
ATOM	1593	O	LEU	A	184	54.017	29.771	31.158	1.00	19.71	O
ATOM	1594	CB	LEU	A	184	52.309	27.553	29.715	1.00	14.41	C
ATOM	1595	CG	LEU	A	184	51.342	27.595	28.525	1.00	23.42	C
ATOM	1596	CD1	LEU	A	184	49.918	27.244	28.928	1.00	31.06	C
ATOM	1597	CD2	LEU	A	184	51.380	28.896	27.690	1.00	21.73	C
ATOM	1598	N	LEU	A	185	55.178	27.879	31.213	1.00	18.39	N
ATOM	1599	CA	LEU	A	185	55.833	28.332	32.417	1.00	16.39	C
ATOM	1600	C	LEU	A	185	56.669	29.528	31.985	1.00	23.67	C
ATOM	1601	O	LEU	A	185	56.681	30.590	32.644	1.00	29.38	O
ATOM	1602	CB	LEU	A	185	56.723	27.233	33.015	1.00	15.05	C
ATOM	1603	CG	LEU	A	185	56.021	26.348	34.041	1.00	15.56	C
ATOM	1604	CD1	LEU	A	185	56.819	25.022	34.301	1.00	21.06	C
ATOM	1605	CD2	LEU	A	185	55.722	27.113	35.321	1.00	11.02	C
ATOM	1606	N	ARG	A	186	57.337	29.397	30.852	1.00	17.09	N
ATOM	1607	CA	ARG	A	186	58.137	30.523	30.429	1.00	18.82	C
ATOM	1608	C	ARG	A	186	57.308	31.752	30.069	1.00	29.00	C
ATOM	1609	O	ARG	A	186	57.629	32.880	30.476	1.00	23.91	O
ATOM	1610	CB	ARG	A	186	59.026	30.146	29.281	1.00	22.06	C
ATOM	1611	CG	ARG	A	186	59.653	31.365	28.652	1.00	38.46	C
ATOM	1612	CD	ARG	A	186	60.825	31.804	29.462	1.00	83.66	C
ATOM	1613	NE	ARG	A	186	62.012	31.861	28.631	1.00	70.77	N
ATOM	1614	CZ	ARG	A	186	63.058	32.622	28.904	1.00	91.68	C
ATOM	1615	NH1	ARG	A	186	63.053	33.386	29.995	1.00	56.56	N
ATOM	1616	NH2	ARG	A	186	64.098	32.639	28.082	1.00	100.00	N
ATOM	1617	N	ARG	A	187	56.234	31.544	29.310	1.00	20.96	N
ATOM	1618	CA	ARG	A	187	55.361	32.662	28.941	1.00	19.32	C
ATOM	1619	C	ARG	A	187	54.765	33.453	30.142	1.00	28.41	C
ATOM	1620	O	ARG	A	187	54.823	34.700	30.193	1.00	17.23	O
ATOM	1621	CB	ARG	A	187	54.270	32.223	27.957	1.00	17.05	C
ATOM	1622	CG	ARG	A	187	54.813	31.546	26.720	1.00	61.42	C
ATOM	1623	CD	ARG	A	187	53.696	31.244	25.757	1.00	44.57	C
ATOM	1624	NE	ARG	A	187	53.033	32.472	25.354	1.00	29.47	N
ATOM	1625	CZ	ARG	A	187	51.831	32.534	24.790	1.00	17.82	C
ATOM	1626	NH1	ARG	A	187	51.136	31.427	24.544	1.00	24.95	N
ATOM	1627	NH2	ARG	A	187	51.341	33.716	24.447	1.00	37.77	N
ATOM	1628	N	PHE	A	188	54.192	32.734	31.101	1.00	23.48	N
ATOM	1629	CA	PHE	A	188	53.604	33.399	32.259	1.00	21.24	C

ATOM	1630	C	PHE	A	188	54.638	34.080	33.095	1.00	21.39	C
ATOM	1631	O	PHE	A	188	54.394	35.126	33.626	1.00	23.90	O
ATOM	1632	CB	PHE	A	188	52.723	32.466	33.077	1.00	19.95	C
ATOM	1633	CG	PHE	A	188	51.389	32.215	32.435	1.00	22.28	C
ATOM	1634	CD1	PHE	A	188	50.440	33.229	32.375	1.00	19.42	C
ATOM	1635	CD2	PHE	A	188	51.144	31.038	31.734	1.00	23.82	C
ATOM	1636	CE1	PHE	A	188	49.191	33.026	31.742	1.00	24.77	C
ATOM	1637	CE2	PHE	A	188	49.936	30.826	31.057	1.00	20.17	C
ATOM	1638	CZ	PHE	A	188	48.945	31.815	31.068	1.00	23.14	C
ATOM	1639	N	HIS	A	189	55.831	33.513	33.118	1.00	24.15	N
ATOM	1640	CA	HIS	A	189	56.933	34.122	33.837	1.00	28.79	C
ATOM	1641	C	HIS	A	189	57.303	35.506	33.315	1.00	28.58	C
ATOM	1642	O	HIS	A	189	57.480	36.463	34.083	1.00	20.07	O
ATOM	1643	CB	HIS	A	189	58.148	33.268	33.641	1.00	31.38	C
ATOM	1644	CG	HIS	A	189	59.364	33.844	34.290	1.00	29.98	C
ATOM	1645	ND1	HIS	A	189	59.548	33.833	35.658	1.00	31.00	N
ATOM	1646	CD2	HIS	A	189	60.449	34.464	33.766	1.00	21.79	C
ATOM	1647	CE1	HIS	A	189	60.722	34.371	35.945	1.00	24.04	C
ATOM	1648	NE2	HIS	A	189	61.257	34.815	34.821	1.00	19.53	N
ATOM	1649	N	GLU	A	190	57.539	35.561	32.006	1.00	28.43	N
ATOM	1650	CA	GLU	A	190	57.876	36.816	31.324	1.00	27.72	C
ATOM	1651	C	GLU	A	190	56.725	37.829	31.437	1.00	32.56	C
ATOM	1652	O	GLU	A	190	56.949	38.995	31.717	1.00	27.06	O
ATOM	1653	CB	GLU	A	190	58.122	36.529	29.849	1.00	28.55	C
ATOM	1654	CG	GLU	A	190	59.150	35.461	29.614	1.00	35.29	C
ATOM	1655	CD	GLU	A	190	60.553	35.941	29.892	1.00	99.81	C
ATOM	1656	OE1	GLU	A	190	60.913	36.037	31.085	1.00	86.56	O
ATOM	1657	OE2	GLU	A	190	61.293	36.167	28.910	1.00	100.00	O
ATOM	1658	N	ALA	A	191	55.493	37.391	31.196	1.00	32.67	N
ATOM	1659	CA	ALA	A	191	54.349	38.286	31.311	1.00	25.30	C
ATOM	1660	C	ALA	A	191	54.287	38.795	32.742	1.00	36.20	C
ATOM	1661	O	ALA	A	191	53.920	39.924	33.014	1.00	27.52	O
ATOM	1662	CB	ALA	A	191	53.055	37.563	31.000	1.00	16.48	C
ATOM	1663	N	THR	A	192	54.549	37.927	33.693	1.00	29.39	N
ATOM	1664	CA	THR	A	192	54.395	38.386	35.041	1.00	19.08	C
ATOM	1665	C	THR	A	192	55.420	39.494	35.298	1.00	44.78	C
ATOM	1666	O	THR	A	192	55.094	40.550	35.839	1.00	40.58	O
ATOM	1667	CB	THR	A	192	54.515	37.235	35.983	1.00	18.99	C
ATOM	1668	OG1	THR	A	192	53.410	36.348	35.755	1.00	34.36	O
ATOM	1669	CG2	THR	A	192	54.461	37.738	37.425	1.00	21.15	C
ATOM	1670	N	ALA	A	193	56.617	39.312	34.757	1.00	48.58	N
ATOM	1671	CA	ALA	A	193	57.705	40.286	34.905	1.00	50.59	C
ATOM	1672	C	ALA	A	193	57.496	41.613	34.145	1.00	54.42	C
ATOM	1673	O	ALA	A	193	57.952	42.698	34.553	1.00	48.28	O
ATOM	1674	CB	ALA	A	193	58.947	39.640	34.496	1.00	51.78	C

ATOM	1675	N	GLN	A	194	56.810	41.530	33.022	1.00	43.16	N
ATOM	1676	CA	GLN	A	194	56.586	42.722	32.242	1.00	38.03	C
ATOM	1677	C	GLN	A	194	55.264	43.389	32.576	1.00	40.85	C
ATOM	1678	O	GLN	A	194	54.830	44.284	31.845	1.00	51.20	O
ATOM	1679	CB	GLN	A	194	56.599	42.358	30.750	1.00	35.96	C
ATOM	1680	CG	GLN	A	194	57.910	41.692	30.290	1.00	100.00	C
ATOM	1681	CD	GLN	A	194	57.715	40.661	29.158	1.00	100.00	C
ATOM	1682	OE1	GLN	A	194	56.619	40.546	28.579	1.00	100.00	O
ATOM	1683	NE2	GLN	A	194	58.782	39.904	28.848	1.00	100.00	N
ATOM	1684	N	GLY	A	195	54.583	42.949	33.630	1.00	32.29	N
ATOM	1685	CA	GLY	A	195	53.236	43.464	33.864	1.00	36.26	C
ATOM	1686	C	GLY	A	195	52.299	43.332	32.593	1.00	45.33	C
ATOM	1687	O	GLY	A	195	51.515	44.242	32.346	1.00	45.16	O
ATOM	1688	N	GLY	A	196	52.405	42.245	31.788	1.00	36.33	N
ATOM	1689	CA	GLY	A	196	51.515	41.965	30.608	1.00	19.06	C
ATOM	1690	C	GLY	A	196	50.037	41.958	31.117	1.00	22.49	C
ATOM	1691	O	GLY	A	196	49.724	41.479	32.223	1.00	33.09	O
ATOM	1692	N	PRO	A	197	49.144	42.657	30.431	1.00	29.22	N
ATOM	1693	CA	PRO	A	197	47.790	42.732	30.953	1.00	25.29	C
ATOM	1694	C	PRO	A	197	47.091	41.413	30.674	1.00	24.64	C
ATOM	1695	O	PRO	A	197	46.192	40.991	31.411	1.00	24.75	O
ATOM	1696	CB	PRO	A	197	47.162	43.911	30.176	1.00	26.31	C
ATOM	1697	CG	PRO	A	197	48.188	44.407	29.252	1.00	26.56	C
ATOM	1698	CD	PRO	A	197	49.307	43.454	29.203	1.00	30.25	C
ATOM	1699	N	ASP	A	198	47.572	40.723	29.658	1.00	16.88	N
ATOM	1700	CA	ASP	A	198	47.067	39.418	29.405	1.00	21.65	C
ATOM	1701	C	ASP	A	198	48.046	38.522	28.677	1.00	31.28	C
ATOM	1702	O	ASP	A	198	49.062	38.978	28.172	1.00	34.57	O
ATOM	1703	CB	ASP	A	198	45.739	39.507	28.669	1.00	32.80	C
ATOM	1704	CG	ASP	A	198	45.868	40.055	27.256	1.00	46.13	C
ATOM	1705	OD1	ASP	A	198	46.982	40.230	26.725	1.00	57.45	O
ATOM	1706	OD2	ASP	A	198	44.817	40.271	26.640	1.00	67.61	O
ATOM	1707	N	VAL	A	199	47.713	37.234	28.614	1.00	38.67	N
ATOM	1708	CA	VAL	A	199	48.499	36.226	27.901	1.00	27.79	C
ATOM	1709	C	VAL	A	199	47.462	35.469	27.065	1.00	25.88	C
ATOM	1710	O	VAL	A	199	46.460	35.023	27.598	1.00	24.22	O
ATOM	1711	CB	VAL	A	199	49.163	35.229	28.905	1.00	24.37	C
ATOM	1712	CG1	VAL	A	199	49.874	34.047	28.160	1.00	20.28	C
ATOM	1713	CG2	VAL	A	199	50.121	35.942	29.835	1.00	22.25	C
ATOM	1714	N	VAL	A	200	47.661	35.386	25.757	1.00	23.72	N
ATOM	1715	CA	VAL	A	200	46.701	34.694	24.903	1.00	23.99	C
ATOM	1716	C	VAL	A	200	47.167	33.286	24.499	1.00	22.85	C
ATOM	1717	O	VAL	A	200	48.321	33.108	24.188	1.00	29.77	O
ATOM	1718	CB	VAL	A	200	46.358	35.548	23.680	1.00	23.11	C
ATOM	1719	CG1	VAL	A	200	45.561	34.737	22.598	1.00	16.25	C

ATOM	1720	CG2	VAL	A	200	45.652	36.823	24.130	1.00	27.86	C
ATOM	1721	N	VAL	A	201	46.296	32.278	24.632	1.00	27.39	N
ATOM	1722	CA	VAL	A	201	46.588	30.893	24.265	1.00	9.63	C
ATOM	1723	C	VAL	A	201	45.653	30.529	23.165	1.00	19.63	C
ATOM	1724	O	VAL	A	201	44.452	30.755	23.312	1.00	17.61	O
ATOM	1725	CB	VAL	A	201	46.306	29.952	25.426	1.00	19.95	C
ATOM	1726	CG1	VAL	A	201	46.703	28.519	25.054	1.00	20.85	C
ATOM	1727	CG2	VAL	A	201	47.086	30.439	26.661	1.00	16.73	C
ATOM	1728	N	TRP	A	202	46.210	30.080	22.030	1.00	14.36	N
ATOM	1729	CA	TRP	A	202	45.422	29.693	20.865	1.00	18.97	C
ATOM	1730	C	TRP	A	202	44.495	28.572	21.313	1.00	36.22	C
ATOM	1731	O	TRP	A	202	44.934	27.694	22.057	1.00	31.46	O
ATOM	1732	CB	TRP	A	202	46.292	29.055	19.823	1.00	19.14	C
ATOM	1733	CG	TRP	A	202	47.243	29.894	19.066	1.00	33.65	C
ATOM	1734	CD1	TRP	A	202	48.391	29.463	18.429	1.00	35.28	C
ATOM	1735	CD2	TRP	A	202	47.126	31.282	18.772	1.00	39.90	C
ATOM	1736	NE1	TRP	A	202	48.941	30.481	17.693	1.00	37.86	N
ATOM	1737	CE2	TRP	A	202	48.228	31.624	17.922	1.00	38.35	C
ATOM	1738	CE3	TRP	A	202	46.206	32.281	19.138	1.00	39.39	C
ATOM	1739	CZ2	TRP	A	202	48.380	32.884	17.367	1.00	36.15	C
ATOM	1740	CZ3	TRP	A	202	46.356	33.542	18.578	1.00	39.60	C
ATOM	1741	CH2	TRP	A	202	47.428	33.828	17.684	1.00	40.99	C
ATOM	1742	N	GLY	A	203	43.245	28.564	20.842	1.00	25.59	N
ATOM	1743	CA	GLY	A	203	42.332	27.483	21.169	1.00	13.09	C
ATOM	1744	C	GLY	A	203	41.260	27.813	22.193	1.00	21.12	C
ATOM	1745	O	GLY	A	203	41.340	28.815	22.886	1.00	22.86	O
ATOM	1746	N	SER	A	204	40.270	26.919	22.262	1.00	16.88	N
ATOM	1747	CA	SER	A	204	39.163	26.979	23.192	1.00	18.36	C
ATOM	1748	C	SER	A	204	39.561	26.664	24.659	1.00	22.07	C
ATOM	1749	O	SER	A	204	38.888	27.096	25.604	1.00	34.39	O
ATOM	1750	CB	SER	A	204	38.053	25.998	22.740	1.00	9.99	C
ATOM	1751	OG	SER	A	204	38.237	24.695	23.291	1.00	16.37	O
ATOM	1752	N	GLY	A	205	40.562	25.813	24.854	1.00	12.42	N
ATOM	1753	CA	GLY	A	205	40.963	25.411	26.208	1.00	11.64	C
ATOM	1754	C	GLY	A	205	40.208	24.178	26.711	1.00	19.49	C
ATOM	1755	O	GLY	A	205	40.422	23.723	27.838	1.00	13.59	O
ATOM	1756	N	THR	A	206	39.292	23.683	25.881	1.00	15.38	N
ATOM	1757	CA	THR	A	206	38.432	22.594	26.281	1.00	10.80	C
ATOM	1758	C	THR	A	206	39.056	21.221	26.154	1.00	26.39	C
ATOM	1759	O	THR	A	206	38.564	20.267	26.737	1.00	23.28	O
ATOM	1760	CB	THR	A	206	37.124	22.562	25.460	1.00	12.86	C
ATOM	1761	OG1	THR	A	206	37.438	22.395	24.082	1.00	13.12	O
ATOM	1762	CG2	THR	A	206	36.348	23.840	25.620	1.00	10.62	C
ATOM	1763	N	PRO	A	207	40.101	21.083	25.354	1.00	21.10	N
ATOM	1764	CA	PRO	A	207	40.658	19.743	25.175	1.00	18.15	C

ATOM	1765	C	PRO	A	207	41.316	19.181	26.423	1.00	21.75	C
ATOM	1766	O	PRO	A	207	41.951	19.925	27.215	1.00	20.65	O
ATOM	1767	CB	PRO	A	207	41.638	19.909	24.013	1.00	17.51	C
ATOM	1768	CG	PRO	A	207	41.146	21.213	23.307	1.00	21.45	C
ATOM	1769	CD	PRO	A	207	40.698	22.062	24.431	1.00	23.44	C
ATOM	1770	N	MET	A	208	41.112	17.876	26.624	1.00	15.60	N
ATOM	1771	CA	MET	A	208	41.694	17.167	27.775	1.00	22.94	C
ATOM	1772	C	MET	A	208	43.058	16.427	27.579	1.00	21.90	C
ATOM	1773	O	MET	A	208	43.248	15.677	26.633	1.00	23.16	O
ATOM	1774	CB	MET	A	208	40.645	16.273	28.386	1.00	32.86	C
ATOM	1775	CG	MET	A	208	39.630	17.057	29.223	1.00	46.17	C
ATOM	1776	SD	MET	A	208	38.301	15.990	29.826	1.00	57.85	S
ATOM	1777	CE	MET	A	208	37.999	15.028	28.343	1.00	58.23	C
ATOM	1778	N	ARG	A	209	44.022	16.681	28.456	1.00	17.75	N
ATOM	1779	CA	ARG	A	209	45.318	16.042	28.324	1.00	19.88	C
ATOM	1780	C	ARG	A	209	45.871	15.534	29.639	1.00	16.92	C
ATOM	1781	O	ARG	A	209	45.433	15.946	30.697	1.00	16.58	O
ATOM	1782	CB	ARG	A	209	46.340	16.963	27.658	1.00	21.07	C
ATOM	1783	CG	ARG	A	209	45.980	17.478	26.275	1.00	22.57	C
ATOM	1784	CD	ARG	A	209	45.833	16.357	25.282	1.00	28.26	C
ATOM	1785	NE	ARG	A	209	45.586	16.819	23.906	1.00	23.15	N
ATOM	1786	CZ	ARG	A	209	44.420	16.742	23.267	1.00	34.52	C
ATOM	1787	NH1	ARG	A	209	43.336	16.267	23.890	1.00	18.03	N
ATOM	1788	NH2	ARG	A	209	44.339	17.175	22.012	1.00	29.78	N
ATOM	1789	N	GLU	A	210	46.878	14.675	29.547	1.00	20.87	N
ATOM	1790	CA	GLU	A	210	47.530	14.079	30.720	1.00	17.37	C
ATOM	1791	C	GLU	A	210	49.031	14.490	30.851	1.00	20.96	C
ATOM	1792	O	GLU	A	210	49.748	14.622	29.841	1.00	22.44	O
ATOM	1793	CB	GLU	A	210	47.400	12.562	30.571	1.00	16.26	C
ATOM	1794	CG	GLU	A	210	47.807	11.785	31.809	1.00	19.91	C
ATOM	1795	CD	GLU	A	210	48.057	10.304	31.531	1.00	27.81	C
ATOM	1796	OE1	GLU	A	210	48.111	9.919	30.343	1.00	17.29	O
ATOM	1797	OE2	GLU	A	210	48.268	9.540	32.494	1.00	21.63	O
ATOM	1798	N	PHE	A	211	49.504	14.712	32.084	1.00	14.02	N
ATOM	1799	CA	PHE	A	211	50.887	15.159	32.353	1.00	17.48	C
ATOM	1800	C	PHE	A	211	51.458	14.414	33.531	1.00	33.62	C
ATOM	1801	O	PHE	A	211	50.716	14.031	34.443	1.00	27.96	O
ATOM	1802	CB	PHE	A	211	50.933	16.677	32.644	1.00	17.78	C
ATOM	1803	CG	PHE	A	211	50.303	17.490	31.541	1.00	21.49	C
ATOM	1804	CD1	PHE	A	211	51.009	17.676	30.320	1.00	17.36	C
ATOM	1805	CD2	PHE	A	211	48.933	17.844	31.618	1.00	15.09	C
ATOM	1806	CE1	PHE	A	211	50.399	18.334	29.237	1.00	16.37	C
ATOM	1807	CE2	PHE	A	211	48.288	18.491	30.533	1.00	9.61	C
ATOM	1808	CZ	PHE	A	211	49.053	18.756	29.344	1.00	12.71	C
ATOM	1809	N	LEU	A	212	52.761	14.161	33.495	1.00	23.76	N

ATOM	1810	CA	LEU	A	212	53.405	13.448	34.603	1.00	21.24	C
ATOM	1811	C	LEU	A	212	54.772	14.053	34.898	1.00	14.00	C
ATOM	1812	O	LEU	A	212	55.519	14.398	33.985	1.00	13.99	O
ATOM	1813	CB	LEU	A	212	53.548	11.954	34.294	1.00	21.52	C
ATOM	1814	CG	LEU	A	212	54.033	11.039	35.406	1.00	21.09	C
ATOM	1815	CD1	LEU	A	212	52.866	10.634	36.280	1.00	20.84	C
ATOM	1816	CD2	LEU	A	212	54.768	9.829	34.832	1.00	13.18	C
ATOM	1817	N	HIS	A	213	55.023	14.302	36.175	1.00	9.60	N
ATOM	1818	CA	HIS	A	213	56.290	14.864	36.555	1.00	13.66	C
ATOM	1819	C	HIS	A	213	57.380	13.828	36.293	1.00	20.37	C
ATOM	1820	O	HIS	A	213	57.238	12.614	36.542	1.00	16.08	O
ATOM	1821	CB	HIS	A	213	56.280	15.250	38.002	1.00	18.72	C
ATOM	1822	CG	HIS	A	213	57.491	16.017	38.408	1.00	21.22	C
ATOM	1823	ND1	HIS	A	213	58.703	15.406	38.656	1.00	24.29	N
ATOM	1824	CD2	HIS	A	213	57.716	17.353	38.499	1.00	23.67	C
ATOM	1825	CE1	HIS	A	213	59.615	16.331	38.917	1.00	19.13	C
ATOM	1826	NE2	HIS	A	213	59.041	17.523	38.847	1.00	21.99	N
ATOM	1827	N	VAL	A	214	58.459	14.295	35.698	1.00	21.07	N
ATOM	1828	CA	VAL	A	214	59.532	13.383	35.361	1.00	19.23	C
ATOM	1829	C	VAL	A	214	60.067	12.523	36.551	1.00	27.20	C
ATOM	1830	O	VAL	A	214	60.604	11.444	36.359	1.00	22.23	O
ATOM	1831	CB	VAL	A	214	60.625	14.125	34.566	1.00	11.84	C
ATOM	1832	CG1	VAL	A	214	61.390	15.199	35.485	1.00	8.52	C
ATOM	1833	CG2	VAL	A	214	61.560	13.097	33.902	1.00	12.39	C
ATOM	1834	N	ASP	A	215	59.893	12.984	37.790	1.00	25.29	N
ATOM	1835	CA	ASP	A	215	60.406	12.228	38.936	1.00	18.19	C
ATOM	1836	C	ASP	A	215	59.530	11.023	39.230	1.00	13.85	C
ATOM	1837	O	ASP	A	215	59.988	9.981	39.666	1.00	17.44	O
ATOM	1838	CB	ASP	A	215	60.575	13.129	40.155	1.00	16.27	C
ATOM	1839	CG	ASP	A	215	61.859	13.979	40.068	1.00	30.73	C
ATOM	1840	OD1	ASP	A	215	62.782	13.614	39.308	1.00	23.02	O
ATOM	1841	OD2	ASP	A	215	61.957	15.029	40.730	1.00	26.00	O
ATOM	1842	N	ASP	A	216	58.276	11.136	38.863	1.00	20.08	N
ATOM	1843	CA	ASP	A	216	57.378	10.017	39.016	1.00	18.78	C
ATOM	1844	C	ASP	A	216	57.761	9.083	37.894	1.00	23.56	C
ATOM	1845	O	ASP	A	216	57.715	7.880	38.026	1.00	20.79	O
ATOM	1846	CB	ASP	A	216	55.912	10.457	38.821	1.00	17.18	C
ATOM	1847	CG	ASP	A	216	55.193	10.757	40.162	1.00	38.03	C
ATOM	1848	OD1	ASP	A	216	55.503	10.119	41.223	1.00	26.02	O
ATOM	1849	OD2	ASP	A	216	54.249	11.587	40.124	1.00	25.41	O
ATOM	1850	N	MET	A	217	58.092	9.653	36.755	1.00	18.11	N
ATOM	1851	CA	MET	A	217	58.394	8.785	35.636	1.00	22.41	C
ATOM	1852	C	MET	A	217	59.572	7.942	35.992	1.00	27.54	C
ATOM	1853	O	MET	A	217	59.579	6.752	35.710	1.00	20.86	O
ATOM	1854	CB	MET	A	217	58.637	9.592	34.345	1.00	21.24	C

ATOM	1855	CG	MET	A	217	59.478	8.918	33.287	1.00	16.37	C
ATOM	1856	SD	MET	A	217	58.962	7.412	32.473	1.00	30.51	S
ATOM	1857	CE	MET	A	217	57.465	7.608	32.391	1.00	19.57	C
ATOM	1858	N	ALA	A	218	60.561	8.562	36.623	1.00	19.09	N
ATOM	1859	CA	ALA	A	218	61.774	7.841	37.002	1.00	13.65	C
ATOM	1860	C	ALA	A	218	61.436	6.778	38.028	1.00	22.61	C
ATOM	1861	O	ALA	A	218	61.934	5.670	37.967	1.00	19.36	O
ATOM	1862	CB	ALA	A	218	62.809	8.780	37.579	1.00	12.23	C
ATOM	1863	N	ALA	A	219	60.605	7.109	39.000	1.00	19.34	N
ATOM	1864	CA	ALA	A	219	60.310	6.105	40.023	1.00	18.01	C
ATOM	1865	C	ALA	A	219	59.630	4.901	39.413	1.00	23.57	C
ATOM	1866	O	ALA	A	219	59.781	3.777	39.898	1.00	22.71	O
ATOM	1867	CB	ALA	A	219	59.387	6.678	41.083	1.00	10.11	C
ATOM	1868	N	ALA	A	220	58.753	5.174	38.454	1.00	18.99	N
ATOM	1869	CA	ALA	A	220	57.905	4.158	37.855	1.00	14.12	C
ATOM	1870	C	ALA	A	220	58.753	3.213	37.034	1.00	25.33	C
ATOM	1871	O	ALA	A	220	58.584	2.006	37.114	1.00	20.63	O
ATOM	1872	CB	ALA	A	220	56.796	4.798	37.023	1.00	8.53	C
ATOM	1873	N	SER	A	221	59.770	3.772	36.379	1.00	23.92	N
ATOM	1874	CA	SER	A	221	60.702	3.011	35.556	1.00	18.38	C
ATOM	1875	C	SER	A	221	61.537	1.989	36.353	1.00	20.90	C
ATOM	1876	O	SER	A	221	61.683	0.799	35.983	1.00	19.84	O
ATOM	1877	CB	SER	A	221	61.604	3.985	34.804	1.00	10.67	C
ATOM	1878	OG	SER	A	221	60.847	4.744	33.867	1.00	15.61	O
ATOM	1879	N	ILE	A	222	62.083	2.476	37.463	1.00	18.12	N
ATOM	1880	CA	ILE	A	222	62.866	1.644	38.381	1.00	21.56	C
ATOM	1881	C	ILE	A	222	62.020	0.554	39.068	1.00	29.10	C
ATOM	1882	O	ILE	A	222	62.504	-0.566	39.307	1.00	19.03	O
ATOM	1883	CB	ILE	A	222	63.467	2.516	39.432	1.00	24.56	C
ATOM	1884	CG1	ILE	A	222	64.465	3.473	38.765	1.00	32.13	C
ATOM	1885	CG2	ILE	A	222	64.129	1.671	40.500	1.00	28.26	C
ATOM	1886	CD1	ILE	A	222	64.973	4.585	39.649	1.00	15.61	C
ATOM	1887	N	HIS	A	223	60.772	0.907	39.384	1.00	19.34	N
ATOM	1888	CA	HIS	A	223	59.829	-0.031	39.996	1.00	20.46	C
ATOM	1889	C	HIS	A	223	59.599	-1.097	38.964	1.00	24.82	C
ATOM	1890	O	HIS	A	223	59.723	-2.283	39.270	1.00	24.66	O
ATOM	1891	CB	HIS	A	223	58.465	0.637	40.359	1.00	19.53	C
ATOM	1892	CG	HIS	A	223	57.373	-0.333	40.759	1.00	28.64	C
ATOM	1893	ND1	HIS	A	223	57.021	-0.564	42.082	1.00	24.16	N
ATOM	1894	CD2	HIS	A	223	56.497	-1.062	40.004	1.00	30.39	C
ATOM	1895	CE1	HIS	A	223	55.983	-1.399	42.112	1.00	30.39	C
ATOM	1896	NE2	HIS	A	223	55.652	-1.727	40.869	1.00	28.13	N
ATOM	1897	N	VAL	A	224	59.354	-0.684	37.725	1.00	22.06	N
ATOM	1898	CA	VAL	A	224	59.111	-1.657	36.652	1.00	19.15	C
ATOM	1899	C	VAL	A	224	60.350	-2.490	36.333	1.00	25.89	C

ATOM	1900	O	VAL	A	224	60.282	-3.709	36.250	1.00	22.37	O
ATOM	1901	CB	VAL	A	224	58.559	-1.022	35.377	1.00	22.59	C
ATOM	1902	CG1	VAL	A	224	58.512	-2.050	34.231	1.00	22.61	C
ATOM	1903	CG2	VAL	A	224	57.161	-0.491	35.650	1.00	23.44	C
ATOM	1904	N	MET	A	225	61.499	-1.838	36.255	1.00	27.83	N
ATOM	1905	CA	MET	A	225	62.710	-2.577	36.004	1.00	23.69	C
ATOM	1906	C	MET	A	225	62.896	-3.678	37.071	1.00	31.95	C
ATOM	1907	O	MET	A	225	63.290	-4.805	36.785	1.00	24.33	O
ATOM	1908	CB	MET	A	225	63.902	-1.604	36.056	1.00	21.34	C
ATOM	1909	CG	MET	A	225	65.295	-2.296	35.999	1.00	17.83	C
ATOM	1910	SD	MET	A	225	65.750	-2.958	34.306	1.00	23.33	S
ATOM	1911	CE	MET	A	225	67.080	-1.896	33.785	1.00	16.46	C
ATOM	1912	N	GLU	A	226	62.644	-3.319	38.316	1.00	19.54	N
ATOM	1913	CA	GLU	A	226	62.988	-4.161	39.428	1.00	21.58	C
ATOM	1914	C	GLU	A	226	61.999	-5.200	39.918	1.00	30.77	C
ATOM	1915	O	GLU	A	226	62.308	-6.012	40.780	1.00	29.39	O
ATOM	1916	CB	GLU	A	226	63.613	-3.323	40.547	1.00	20.47	C
ATOM	1917	CG	GLU	A	226	64.937	-2.673	40.122	1.00	23.03	C
ATOM	1918	CD	GLU	A	226	65.504	-1.809	41.208	1.00	32.62	C
ATOM	1919	OE1	GLU	A	226	64.721	-1.455	42.122	1.00	26.12	O
ATOM	1920	OE2	GLU	A	226	66.711	-1.479	41.152	1.00	17.67	O
ATOM	1921	N	LEU	A	227	60.837	-5.248	39.295	1.00	34.11	N
ATOM	1922	CA	LEU	A	227	59.883	-6.296	39.642	1.00	35.26	C
ATOM	1923	C	LEU	A	227	60.537	-7.644	39.320	1.00	27.91	C
ATOM	1924	O	LEU	A	227	61.291	-7.766	38.340	1.00	19.89	O
ATOM	1925	CB	LEU	A	227	58.693	-6.236	38.678	1.00	36.48	C
ATOM	1926	CG	LEU	A	227	57.381	-5.569	38.955	1.00	40.30	C
ATOM	1927	CD1	LEU	A	227	57.697	-4.194	39.382	1.00	42.04	C
ATOM	1928	CD2	LEU	A	227	56.610	-5.577	37.647	1.00	46.21	C
ATOM	1929	N	ALA	A	228	60.026	-8.688	39.955	1.00	27.15	N
ATOM	1930	CA	ALA	A	228	60.425	-10.051	39.616	1.00	25.26	C
ATOM	1931	C	ALA	A	228	59.801	-10.435	38.279	1.00	27.93	C
ATOM	1932	O	ALA	A	228	58.624	-10.093	37.934	1.00	31.26	O
ATOM	1933	CB	ALA	A	228	60.003	-11.052	40.703	1.00	22.05	C
ATOM	1934	N	HIS	A	229	60.624	-11.160	37.539	1.00	27.05	N
ATOM	1935	CA	HIS	A	229	60.275	-11.605	36.222	1.00	24.42	C
ATOM	1936	C	HIS	A	229	58.905	-12.260	36.184	1.00	21.74	C
ATOM	1937	O	HIS	A	229	58.015	-11.851	35.398	1.00	22.22	O
ATOM	1938	CB	HIS	A	229	61.351	-12.520	35.698	1.00	17.71	C
ATOM	1939	CG	HIS	A	229	61.284	-12.701	34.220	1.00	27.24	C
ATOM	1940	ND1	HIS	A	229	61.060	-11.650	33.350	1.00	34.38	N
ATOM	1941	CD2	HIS	A	229	61.292	-13.821	33.465	1.00	31.45	C
ATOM	1942	CE1	HIS	A	229	60.992	-12.113	32.115	1.00	30.50	C
ATOM	1943	NE2	HIS	A	229	61.124	-13.427	32.159	1.00	35.23	N
ATOM	1944	N	GLU	A	230	58.681	-13.161	37.140	1.00	20.24	N

ATOM	1945	CA	GLU	A	230	57.425	-13.895	37.209	1.00	29.41	C
ATOM	1946	C	GLU	A	230	56.181	-13.051	37.341	1.00	22.20	C
ATOM	1947	O	GLU	A	230	55.159	-13.359	36.679	1.00	17.78	O
ATOM	1948	CB	GLU	A	230	57.464	-14.997	38.274	1.00	38.51	C
ATOM	1949	CG	GLU	A	230	58.085	-14.582	39.567	1.00	63.09	C
ATOM	1950	CD	GLU	A	230	57.036	-14.473	40.661	1.00	100.00	C
ATOM	1951	OE1	GLU	A	230	55.859	-14.872	40.400	1.00	100.00	O
ATOM	1952	OE2	GLU	A	230	57.409	-14.003	41.768	1.00	81.48	O
ATOM	1953	N	VAL	A	231	56.272	-12.004	38.182	1.00	16.53	N
ATOM	1954	CA	VAL	A	231	55.202	-11.029	38.356	1.00	20.23	C
ATOM	1955	C	VAL	A	231	55.009	-10.164	37.102	1.00	24.45	C
ATOM	1956	O	VAL	A	231	53.864	-9.834	36.705	1.00	21.00	O
ATOM	1957	CB	VAL	A	231	55.541	-10.057	39.426	1.00	28.61	C
ATOM	1958	CG1	VAL	A	231	54.362	-9.098	39.610	1.00	29.78	C
ATOM	1959	CG2	VAL	A	231	55.881	-10.757	40.677	1.00	28.96	C
ATOM	1960	N	TRP	A	232	56.133	-9.798	36.486	1.00	17.17	N
ATOM	1961	CA	TRP	A	232	56.052	-9.044	35.262	1.00	21.52	C
ATOM	1962	C	TRP	A	232	55.388	-9.844	34.156	1.00	20.53	C
ATOM	1963	O	TRP	A	232	54.588	-9.306	33.380	1.00	24.31	O
ATOM	1964	CB	TRP	A	232	57.438	-8.644	34.801	1.00	29.88	C
ATOM	1965	CG	TRP	A	232	57.430	-7.843	33.500	1.00	27.65	C
ATOM	1966	CD1	TRP	A	232	57.184	-6.464	33.356	1.00	25.42	C
ATOM	1967	CD2	TRP	A	232	57.714	-8.336	32.169	1.00	27.75	C
ATOM	1968	NE1	TRP	A	232	57.325	-6.095	32.033	1.00	22.53	N
ATOM	1969	CE2	TRP	A	232	57.655	-7.203	31.279	1.00	25.11	C
ATOM	1970	CE3	TRP	A	232	58.037	-9.603	31.640	1.00	22.72	C
ATOM	1971	CZ2	TRP	A	232	57.917	-7.316	29.879	1.00	17.23	C
ATOM	1972	CZ3	TRP	A	232	58.238	-9.720	30.223	1.00	25.97	C
ATOM	1973	CH2	TRP	A	232	58.154	-8.581	29.368	1.00	22.07	C
ATOM	1974	N	LEU	A	233	55.749	-11.121	34.018	1.00	23.80	N
ATOM	1975	CA	LEU	A	233	55.141	-11.949	32.937	1.00	24.78	C
ATOM	1976	C	LEU	A	233	53.652	-12.118	33.122	1.00	24.51	C
ATOM	1977	O	LEU	A	233	52.865	-12.075	32.163	1.00	28.50	O
ATOM	1978	CB	LEU	A	233	55.765	-13.348	32.820	1.00	26.20	C
ATOM	1979	CG	LEU	A	233	57.250	-13.505	32.503	1.00	19.39	C
ATOM	1980	CD1	LEU	A	233	57.745	-14.850	33.023	1.00	19.90	C
ATOM	1981	CD2	LEU	A	233	57.561	-13.287	31.017	1.00	16.01	C
ATOM	1982	N	GLU	A	234	53.298	-12.343	34.372	1.00	25.45	N
ATOM	1983	CA	GLU	A	234	51.929	-12.523	34.822	1.00	30.04	C
ATOM	1984	C	GLU	A	234	51.128	-11.319	34.367	1.00	35.69	C
ATOM	1985	O	GLU	A	234	49.926	-11.390	34.052	1.00	28.25	O
ATOM	1986	CB	GLU	A	234	52.007	-12.468	36.344	1.00	37.30	C
ATOM	1987	CG	GLU	A	234	50.908	-13.133	37.118	1.00	45.39	C
ATOM	1988	CD	GLU	A	234	51.112	-12.881	38.601	1.00	100.00	C
ATOM	1989	OE1	GLU	A	234	52.240	-13.137	39.104	1.00	99.09	O

ATOM	1990	OE2	GLU	A	234	50.211	-12.257	39.211	1.00	100.00	O
ATOM	1991	N	ASN	A	235	51.802	-10.184	34.364	1.00	25.04	N
ATOM	1992	CA	ASN	A	235	51.109	-8.986	33.992	1.00	26.17	C
ATOM	1993	C	ASN	A	235	51.280	-8.494	32.571	1.00	30.46	C
ATOM	1994	O	ASN	A	235	50.824	-7.393	32.259	1.00	22.90	O
ATOM	1995	CB	ASN	A	235	51.427	-7.895	34.981	1.00	29.23	C
ATOM	1996	CG	ASN	A	235	50.878	-8.197	36.342	1.00	39.27	C
ATOM	1997	OD1	ASN	A	235	49.722	-7.882	36.628	1.00	29.06	O
ATOM	1998	ND2	ASN	A	235	51.653	-8.934	37.140	1.00	40.22	N
ATOM	1999	N	THR	A	236	51.935	-9.268	31.708	1.00	20.97	N
ATOM	2000	CA	THR	A	236	52.108	-8.795	30.344	1.00	22.30	C
ATOM	2001	C	THR	A	236	51.867	-9.943	29.419	1.00	29.74	C
ATOM	2002	O	THR	A	236	51.551	-11.033	29.895	1.00	21.23	O
ATOM	2003	CB	THR	A	236	53.545	-8.306	30.161	1.00	22.73	C
ATOM	2004	OG1	THR	A	236	54.422	-9.325	30.636	1.00	21.23	O
ATOM	2005	CG2	THR	A	236	53.801	-7.048	31.041	1.00	19.69	C
ATOM	2006	N	GLN	A	237	52.003	-9.699	28.109	1.00	22.23	N
ATOM	2007	CA	GLN	A	237	52.097	-10.783	27.122	1.00	16.69	C
ATOM	2008	C	GLN	A	237	53.335	-10.507	26.331	1.00	21.02	C
ATOM	2009	O	GLN	A	237	53.729	-9.362	26.204	1.00	22.19	O
ATOM	2010	CB	GLN	A	237	50.913	-10.999	26.189	1.00	8.23	C
ATOM	2011	CG	GLN	A	237	49.639	-11.096	26.904	1.00	21.04	C
ATOM	2012	CD	GLN	A	237	48.907	-9.862	26.606	1.00	62.07	C
ATOM	2013	OE1	GLN	A	237	48.437	-9.712	25.460	1.00	59.32	O
ATOM	2014	NE2	GLN	A	237	49.220	-8.847	27.388	1.00	37.82	N
ATOM	2015	N	PRO	A	238	54.002	-11.579	25.917	1.00	28.76	N
ATOM	2016	CA	PRO	A	238	55.275	-11.438	25.246	1.00	30.28	C
ATOM	2017	C	PRO	A	238	55.194	-10.643	23.958	1.00	29.08	C
ATOM	2018	O	PRO	A	238	56.181	-10.029	23.600	1.00	15.95	O
ATOM	2019	CB	PRO	A	238	55.733	-12.879	25.011	1.00	22.54	C
ATOM	2020	CG	PRO	A	238	54.898	-13.710	25.886	1.00	18.92	C
ATOM	2021	CD	PRO	A	238	53.626	-12.998	26.068	1.00	11.75	C
ATOM	2022	N	MET	A	239	54.041	-10.635	23.286	1.00	17.26	N
ATOM	2023	CA	MET	A	239	53.924	-9.807	22.104	1.00	17.85	C
ATOM	2024	C	MET	A	239	53.109	-8.509	22.362	1.00	18.63	C
ATOM	2025	O	MET	A	239	52.792	-7.741	21.419	1.00	16.82	O
ATOM	2026	CB	MET	A	239	53.460	-10.588	20.881	1.00	15.22	C
ATOM	2027	CG	MET	A	239	54.536	-11.534	20.261	1.00	12.90	C
ATOM	2028	SD	MET	A	239	53.994	-12.534	18.808	1.00	17.49	S
ATOM	2029	CE	MET	A	239	54.350	-11.357	17.422	1.00	13.12	C
ATOM	2030	N	LEU	A	240	52.847	-8.252	23.646	1.00	18.55	N
ATOM	2031	CA	LEU	A	240	52.159	-7.037	24.131	1.00	16.68	C
ATOM	2032	C	LEU	A	240	52.774	-6.733	25.493	1.00	11.82	C
ATOM	2033	O	LEU	A	240	52.124	-6.803	26.549	1.00	13.84	O
ATOM	2034	CB	LEU	A	240	50.645	-7.249	24.240	1.00	16.91	C

ATOM	2035	CG	LEU	A	240	49.646	-6.120	23.852	1.00	22.29	C
ATOM	2036	CD1	LEU	A	240	48.968	-5.488	25.033	1.00	25.51	C
ATOM	2037	CD2	LEU	A	240	50.070	-5.059	22.815	1.00	28.07	C
ATOM	2038	N	SER	A	241	54.076	-6.467	25.456	1.00	13.09	N
ATOM	2039	CA	SER	A	241	54.842	-6.315	26.682	1.00	24.20	C
ATOM	2040	C	SER	A	241	54.947	-4.938	27.377	1.00	30.52	C
ATOM	2041	O	SER	A	241	55.363	-4.854	28.547	1.00	17.02	O
ATOM	2042	CB	SER	A	241	56.247	-6.900	26.495	1.00	14.04	C
ATOM	2043	OG	SER	A	241	57.062	-6.144	25.598	1.00	13.95	O
ATOM	2044	N	HIS	A	242	54.661	-3.861	26.659	1.00	17.87	N
ATOM	2045	CA	HIS	A	242	54.894	-2.548	27.221	1.00	13.55	C
ATOM	2046	C	HIS	A	242	53.990	-2.254	28.373	1.00	13.70	C
ATOM	2047	O	HIS	A	242	52.974	-2.885	28.539	1.00	13.29	O
ATOM	2048	CB	HIS	A	242	54.826	-1.430	26.130	1.00	16.05	C
ATOM	2049	CG	HIS	A	242	53.595	-1.504	25.272	1.00	18.88	C
ATOM	2050	ND1	HIS	A	242	52.591	-0.553	25.326	1.00	23.24	N
ATOM	2051	CD2	HIS	A	242	53.165	-2.461	24.413	1.00	13.19	C
ATOM	2052	CE1	HIS	A	242	51.629	-0.887	24.483	1.00	17.44	C
ATOM	2053	NE2	HIS	A	242	51.962	-2.031	23.901	1.00	19.54	N
ATOM	2054	N	ILE	A	243	54.310	-1.203	29.095	1.00	15.84	N
ATOM	2055	CA	ILE	A	243	53.492	-0.809	30.192	1.00	19.10	C
ATOM	2056	C	ILE	A	243	53.336	0.714	30.191	1.00	23.23	C
ATOM	2057	O	ILE	A	243	54.312	1.406	30.385	1.00	12.10	O
ATOM	2058	CB	ILE	A	243	54.166	-1.273	31.482	1.00	24.62	C
ATOM	2059	CG1	ILE	A	243	54.014	-2.783	31.576	1.00	25.60	C
ATOM	2060	CG2	ILE	A	243	53.497	-0.665	32.735	1.00	17.37	C
ATOM	2061	CD1	ILE	A	243	54.725	-3.365	32.714	1.00	14.82	C
ATOM	2062	N	ASN	A	244	52.112	1.217	30.013	1.00	16.43	N
ATOM	2063	CA	ASN	A	244	51.824	2.689	30.038	1.00	18.99	C
ATOM	2064	C	ASN	A	244	52.252	3.292	31.348	1.00	18.83	C
ATOM	2065	O	ASN	A	244	51.965	2.727	32.405	1.00	19.58	O
ATOM	2066	CB	ASN	A	244	50.304	2.987	29.910	1.00	15.67	C
ATOM	2067	CG	ASN	A	244	49.768	2.702	28.517	1.00	14.57	C
ATOM	2068	OD1	ASN	A	244	50.546	2.583	27.580	1.00	13.64	O
ATOM	2069	ND2	ASN	A	244	48.443	2.491	28.393	1.00	10.16	N
ATOM	2070	N	VAL	A	245	52.800	4.499	31.326	1.00	13.50	N
ATOM	2071	CA	VAL	A	245	53.159	5.134	32.602	1.00	13.49	C
ATOM	2072	C	VAL	A	245	52.528	6.566	32.644	1.00	16.25	C
ATOM	2073	O	VAL	A	245	52.786	7.405	31.770	1.00	15.20	O
ATOM	2074	CB	VAL	A	245	54.754	5.163	32.810	1.00	21.07	C
ATOM	2075	CG1	VAL	A	245	55.154	6.085	33.937	1.00	15.08	C
ATOM	2076	CG2	VAL	A	245	55.280	3.817	33.143	1.00	15.82	C
ATOM	2077	N	GLY	A	246	51.696	6.843	33.649	1.00	14.03	N
ATOM	2078	CA	GLY	A	246	51.027	8.136	33.707	1.00	16.87	C
ATOM	2079	C	GLY	A	246	50.146	8.203	34.939	1.00	26.95	C

ATOM	2080	O	GLY	A	246	50.323	7.401	35.850	1.00	23.04	O
ATOM	2081	N	THR	A	247	49.207	9.161	34.963	1.00	21.44	N
ATOM	2082	CA	THR	A	247	48.232	9.276	36.063	1.00	21.39	C
ATOM	2083	C	THR	A	247	46.868	8.677	35.673	1.00	24.08	C
ATOM	2084	O	THR	A	247	46.069	8.306	36.508	1.00	21.03	O
ATOM	2085	CB	THR	A	247	47.988	10.730	36.404	1.00	22.24	C
ATOM	2086	OG1	THR	A	247	47.409	11.389	35.265	1.00	18.62	O
ATOM	2087	CG2	THR	A	247	49.275	11.378	36.724	1.00	18.99	C
ATOM	2088	N	GLY	A	248	46.583	8.651	34.384	1.00	24.95	N
ATOM	2089	CA	GLY	A	248	45.319	8.143	33.924	1.00	22.61	C
ATOM	2090	C	GLY	A	248	44.223	9.160	34.226	1.00	21.42	C
ATOM	2091	O	GLY	A	248	43.059	8.866	34.137	1.00	25.70	O
ATOM	2092	N	VAL	A	249	44.615	10.386	34.521	1.00	30.72	N
ATOM	2093	CA	VAL	A	249	43.673	11.464	34.827	1.00	26.09	C
ATOM	2094	C	VAL	A	249	43.747	12.596	33.786	1.00	32.70	C
ATOM	2095	O	VAL	A	249	44.853	13.006	33.387	1.00	26.92	O
ATOM	2096	CB	VAL	A	249	44.020	12.085	36.214	1.00	38.59	C
ATOM	2097	CG1	VAL	A	249	43.225	13.324	36.470	1.00	36.11	C
ATOM	2098	CG2	VAL	A	249	43.782	11.083	37.306	1.00	41.30	C
ATOM	2099	N	ASP	A	250	42.581	13.125	33.397	1.00	27.95	N
ATOM	2100	CA	ASP	A	250	42.488	14.232	32.439	1.00	20.64	C
ATOM	2101	C	ASP	A	250	42.611	15.581	33.155	1.00	27.63	C
ATOM	2102	O	ASP	A	250	42.188	15.783	34.308	1.00	26.23	O
ATOM	2103	CB	ASP	A	250	41.075	14.302	31.827	1.00	23.89	C
ATOM	2104	CG	ASP	A	250	40.768	13.180	30.850	1.00	39.52	C
ATOM	2105	OD1	ASP	A	250	41.283	13.184	29.688	1.00	39.96	O
ATOM	2106	OD2	ASP	A	250	39.767	12.501	31.153	1.00	45.34	O
ATOM	2107	N	CYS	A	251	43.029	16.566	32.388	1.00	20.12	N
ATOM	2108	CA	CYS	A	251	42.962	17.906	32.851	1.00	27.20	C
ATOM	2109	C	CYS	A	251	42.918	18.779	31.577	1.00	26.47	C
ATOM	2110	O	CYS	A	251	43.699	18.560	30.633	1.00	19.45	O
ATOM	2111	CB	CYS	A	251	44.148	18.157	33.778	1.00	34.86	C
ATOM	2112	SG	CYS	A	251	45.129	19.619	33.453	1.00	29.47	S
ATOM	2113	N	THR	A	252	41.932	19.673	31.494	1.00	14.85	N
ATOM	2114	CA	THR	A	252	41.834	20.588	30.335	1.00	21.21	C
ATOM	2115	C	THR	A	252	42.999	21.592	30.236	1.00	20.53	C
ATOM	2116	O	THR	A	252	43.657	21.926	31.249	1.00	15.24	O
ATOM	2117	CB	THR	A	252	40.506	21.407	30.329	1.00	32.08	C
ATOM	2118	OG1	THR	A	252	40.460	22.304	31.447	1.00	19.26	O
ATOM	2119	CG2	THR	A	252	39.309	20.495	30.372	1.00	13.91	C
ATOM	2120	N	ILE	A	253	43.228	22.095	29.024	1.00	14.81	N
ATOM	2121	CA	ILE	A	253	44.264	23.118	28.812	1.00	16.90	C
ATOM	2122	C	ILE	A	253	43.934	24.383	29.627	1.00	23.41	C
ATOM	2123	O	ILE	A	253	44.834	25.012	30.247	1.00	15.27	O
ATOM	2124	CB	ILE	A	253	44.404	23.452	27.302	1.00	24.05	C

ATOM	2125	CG1	ILE	A	253	44.862	22.200	26.561	1.00	27.33	C
ATOM	2126	CG2	ILE	A	253	45.473	24.479	27.077	1.00	9.22	C
ATOM	2127	CD1	ILE	A	253	45.662	21.276	27.452	1.00	49.56	C
ATOM	2128	N	ARG	A	254	42.637	24.709	29.707	1.00	19.56	N
ATOM	2129	CA	ARG	A	254	42.228	25.865	30.522	1.00	19.41	C
ATOM	2130	C	ARG	A	254	42.712	25.713	31.970	1.00	18.10	C
ATOM	2131	O	ARG	A	254	43.311	26.616	32.515	1.00	13.89	O
ATOM	2132	CB	ARG	A	254	40.704	26.101	30.480	1.00	15.98	C
ATOM	2133	CG	ARG	A	254	40.282	27.378	31.255	1.00	9.96	C
ATOM	2134	CD	ARG	A	254	38.809	27.702	31.218	1.00	24.79	C
ATOM	2135	NE	ARG	A	254	38.498	28.414	29.997	1.00	29.42	N
ATOM	2136	CZ	ARG	A	254	38.693	29.723	29.794	1.00	59.85	C
ATOM	2137	NH1	ARG	A	254	39.194	30.527	30.732	1.00	42.58	N
ATOM	2138	NH2	ARG	A	254	38.377	30.245	28.620	1.00	18.44	N
ATOM	2139	N	ASP	A	255	42.406	24.564	32.586	1.00	20.22	N
ATOM	2140	CA	ASP	A	255	42.795	24.205	33.974	1.00	16.48	C
ATOM	2141	C	ASP	A	255	44.321	24.372	34.069	1.00	22.43	C
ATOM	2142	O	ASP	A	255	44.868	24.897	35.060	1.00	18.53	O
ATOM	2143	CB	ASP	A	255	42.478	22.686	34.157	1.00	19.17	C
ATOM	2144	CG	ASP	A	255	42.144	22.246	35.610	1.00	47.08	C
ATOM	2145	OD1	ASP	A	255	41.780	23.090	36.429	1.00	49.66	O
ATOM	2146	OD2	ASP	A	255	42.020	21.016	35.880	1.00	48.12	O
ATOM	2147	N	LEU	A	256	45.014	23.809	33.078	1.00	15.98	N
ATOM	2148	CA	LEU	A	256	46.465	23.844	33.069	1.00	21.76	C
ATOM	2149	C	LEU	A	256	47.020	25.275	33.076	1.00	16.79	C
ATOM	2150	O	LEU	A	256	47.825	25.697	33.946	1.00	15.24	O
ATOM	2151	CB	LEU	A	256	46.967	23.056	31.859	1.00	23.33	C
ATOM	2152	CG	LEU	A	256	48.491	23.100	31.765	1.00	26.80	C
ATOM	2153	CD1	LEU	A	256	49.171	22.334	32.984	1.00	17.13	C
ATOM	2154	CD2	LEU	A	256	49.040	22.724	30.346	1.00	15.42	C
ATOM	2155	N	ALA	A	257	46.520	26.048	32.140	1.00	13.77	N
ATOM	2156	CA	ALA	A	257	46.938	27.436	32.025	1.00	12.70	C
ATOM	2157	C	ALA	A	257	46.656	28.237	33.267	1.00	10.73	C
ATOM	2158	O	ALA	A	257	47.451	29.073	33.672	1.00	20.33	O
ATOM	2159	CB	ALA	A	257	46.208	28.073	30.834	1.00	13.34	C
ATOM	2160	N	GLN	A	258	45.470	28.080	33.835	1.00	12.40	N
ATOM	2161	CA	GLN	A	258	45.102	28.911	34.981	1.00	8.39	C
ATOM	2162	C	GLN	A	258	45.879	28.480	36.166	1.00	13.48	C
ATOM	2163	O	GLN	A	258	46.178	29.281	37.029	1.00	22.96	O
ATOM	2164	CB	GLN	A	258	43.614	28.761	35.305	1.00	16.12	C
ATOM	2165	CG	GLN	A	258	42.674	29.096	34.130	1.00	30.19	C
ATOM	2166	CD	GLN	A	258	42.574	30.585	33.781	1.00	37.29	C
ATOM	2167	OE1	GLN	A	258	42.911	31.471	34.610	1.00	21.24	O
ATOM	2168	NE2	GLN	A	258	42.021	30.876	32.572	1.00	15.94	N
ATOM	2169	N	THR	A	259	46.179	27.182	36.232	1.00	16.21	N

ATOM	2170	CA	THR	A	259	46.982	26.678	37.336	1.00	16.85	C
ATOM	2171	C	THR	A	259	48.410	27.186	37.233	1.00	20.56	C
ATOM	2172	O	THR	A	259	49.002	27.621	38.214	1.00	21.44	O
ATOM	2173	CB	THR	A	259	47.066	25.192	37.361	1.00	27.56	C
ATOM	2174	OG1	THR	A	259	45.752	24.620	37.509	1.00	20.92	O
ATOM	2175	CG2	THR	A	259	47.936	24.796	38.545	1.00	12.85	C
ATOM	2176	N	ILE	A	260	48.952	27.170	36.028	1.00	19.96	N
ATOM	2177	CA	ILE	A	260	50.292	27.704	35.839	1.00	23.01	C
ATOM	2178	C	ILE	A	260	50.313	29.180	36.225	1.00	31.73	C
ATOM	2179	O	ILE	A	260	51.211	29.627	36.993	1.00	25.90	O
ATOM	2180	CB	ILE	A	260	50.835	27.456	34.390	1.00	22.46	C
ATOM	2181	CG1	ILE	A	260	51.153	25.940	34.232	1.00	24.12	C
ATOM	2182	CG2	ILE	A	260	52.099	28.361	34.106	1.00	13.47	C
ATOM	2183	CD1	ILE	A	260	51.501	25.443	32.810	1.00	12.58	C
ATOM	2184	N	ALA	A	261	49.280	29.910	35.764	1.00	15.35	N
ATOM	2185	CA	ALA	A	261	49.177	31.355	36.048	1.00	16.00	C
ATOM	2186	C	ALA	A	261	49.316	31.604	37.550	1.00	20.58	C
ATOM	2187	O	ALA	A	261	50.104	32.443	37.987	1.00	16.09	O
ATOM	2188	CB	ALA	A	261	47.832	31.958	35.487	1.00	13.65	C
ATOM	2189	N	LYS	A	262	48.551	30.843	38.323	1.00	11.50	N
ATOM	2190	CA	LYS	A	262	48.578	30.905	39.770	1.00	10.13	C
ATOM	2191	C	LYS	A	262	49.968	30.460	40.296	1.00	28.08	C
ATOM	2192	O	LYS	A	262	50.503	31.084	41.205	1.00	29.37	O
ATOM	2193	CB	LYS	A	262	47.453	30.032	40.335	1.00	12.50	C
ATOM	2194	CG	LYS	A	262	47.332	29.962	41.888	1.00	16.51	C
ATOM	2195	CD	LYS	A	262	46.092	29.092	42.371	1.00	46.61	C
ATOM	2196	CE	LYS	A	262	46.344	27.555	42.661	1.00	99.70	C
ATOM	2197	NZ	LYS	A	262	45.157	26.703	43.200	1.00	36.59	N
ATOM	2198	N	VAL	A	263	50.589	29.443	39.705	1.00	17.44	N
ATOM	2199	CA	VAL	A	263	51.915	29.039	40.171	1.00	18.72	C
ATOM	2200	C	VAL	A	263	52.997	30.170	39.997	1.00	32.12	C
ATOM	2201	O	VAL	A	263	53.871	30.412	40.834	1.00	21.18	O
ATOM	2202	CB	VAL	A	263	52.389	27.709	39.476	1.00	16.35	C
ATOM	2203	CG1	VAL	A	263	53.920	27.518	39.647	1.00	11.83	C
ATOM	2204	CG2	VAL	A	263	51.646	26.522	40.093	1.00	14.99	C
ATOM	2205	N	VAL	A	264	52.913	30.899	38.909	1.00	21.75	N
ATOM	2206	CA	VAL	A	264	53.917	31.877	38.653	1.00	19.81	C
ATOM	2207	C	VAL	A	264	53.719	33.208	39.377	1.00	35.79	C
ATOM	2208	O	VAL	A	264	54.632	34.032	39.482	1.00	28.99	O
ATOM	2209	CB	VAL	A	264	54.059	32.014	37.175	1.00	24.27	C
ATOM	2210	CG1	VAL	A	264	54.728	33.269	36.822	1.00	33.58	C
ATOM	2211	CG2	VAL	A	264	54.840	30.808	36.674	1.00	23.01	C
ATOM	2212	N	GLY	A	265	52.550	33.378	39.969	1.00	25.30	N
ATOM	2213	CA	GLY	A	265	52.241	34.620	40.636	1.00	24.14	C
ATOM	2214	C	GLY	A	265	51.730	35.694	39.632	1.00	35.03	C

ATOM	2215	O	GLY	A	265	51.773	36.911	39.962	1.00 33.71	O
ATOM	2216	N	TYR	A	266	51.294	35.257	38.428	1.00 26.25	N
ATOM	2217	CA	TYR	A	266	50.698	36.151	37.373	1.00 26.55	C
ATOM	2218	C	TYR	A	266	49.364	36.745	37.818	1.00 31.01	C
ATOM	2219	O	TYR	A	266	48.532	36.067	38.456	1.00 27.99	O
ATOM	2220	CB	TYR	A	266	50.501	35.463	36.008	1.00 24.31	C
ATOM	2221	CG	TYR	A	266	49.994	36.381	34.884	1.00 28.64	C
ATOM	2222	CD1	TYR	A	266	50.670	37.582	34.542	1.00 35.05	C
ATOM	2223	CD2	TYR	A	266	48.860	36.038	34.118	1.00 22.60	C
ATOM	2224	CE1	TYR	A	266	50.212	38.434	33.472	1.00 20.73	C
ATOM	2225	CE2	TYR	A	266	48.428	36.859	33.012	1.00 20.91	C
ATOM	2226	CZ	TYR	A	266	49.088	38.062	32.735	1.00 23.85	C
ATOM	2227	OH	TYR	A	266	48.622	38.851	31.710	1.00 33.40	O
ATOM	2228	N	LYS	A	267	49.217	38.043	37.604	1.00 25.72	N
ATOM	2229	CA	LYS	A	267	47.988	38.697	38.009	1.00 30.77	C
ATOM	2230	C	LYS	A	267	47.217	39.280	36.798	1.00 28.85	C
ATOM	2231	O	LYS	A	267	46.179	39.894	36.949	1.00 31.17	O
ATOM	2232	CB	LYS	A	267	48.279	39.741	39.092	1.00 27.13	C
ATOM	2233	CG	LYS	A	267	48.728	39.128	40.403	1.00 23.18	C
ATOM	2234	CD	LYS	A	267	48.420	40.096	41.562	1.00 30.98	C
ATOM	2235	CE	LYS	A	267	47.933	39.358	42.820	1.00 48.52	C
ATOM	2236	NZ	LYS	A	267	47.005	38.208	42.505	1.00100.00	N
ATOM	2237	N	GLY	A	268	47.716	39.054	35.594	1.00 22.67	N
ATOM	2238	CA	GLY	A	268	47.019	39.518	34.394	1.00 21.38	C
ATOM	2239	C	GLY	A	268	45.856	38.568	34.085	1.00 31.03	C
ATOM	2240	O	GLY	A	268	45.455	37.728	34.911	1.00 19.71	O
ATOM	2241	N	ARG	A	269	45.387	38.645	32.849	1.00 30.40	N
ATOM	2242	CA	ARG	A	269	44.263	37.846	32.399	1.00 26.47	C
ATOM	2243	C	ARG	A	269	44.680	36.705	31.489	1.00 22.35	C
ATOM	2244	O	ARG	A	269	45.378	36.926	30.524	1.00 22.75	O
ATOM	2245	CB	ARG	A	269	43.297	38.753	31.626	1.00 22.65	C
ATOM	2246	CG	ARG	A	269	42.201	39.390	32.463	1.00 24.21	C
ATOM	2247	CD	ARG	A	269	40.936	39.465	31.568	1.00 83.45	C
ATOM	2248	NE	ARG	A	269	40.113	40.676	31.762	1.00100.00	N
ATOM	2249	CZ	ARG	A	269	38.808	40.751	31.431	1.00100.00	C
ATOM	2250	NH1	ARG	A	269	38.201	39.691	30.921	1.00 99.93	N
ATOM	2251	NH2	ARG	A	269	38.094	41.865	31.663	1.00100.00	N
ATOM	2252	N	VAL	A	270	44.195	35.494	31.758	1.00 19.87	N
ATOM	2253	CA	VAL	A	270	44.468	34.389	30.856	1.00 24.82	C
ATOM	2254	C	VAL	A	270	43.319	34.456	29.824	1.00 22.51	C
ATOM	2255	O	VAL	A	270	42.145	34.501	30.181	1.00 25.79	O
ATOM	2256	CB	VAL	A	270	44.436	32.979	31.571	1.00 24.03	C
ATOM	2257	CG1	VAL	A	270	44.576	31.861	30.533	1.00 20.72	C
ATOM	2258	CG2	VAL	A	270	45.506	32.849	32.639	1.00 11.27	C
ATOM	2259	N	VAL	A	271	43.660	34.409	28.554	1.00 25.18	N

ATOM	2260	CA	VAL	A	271	42.666	34.492	27.487	1.00	28.32	C
ATOM	2261	C	VAL	A	271	42.819	33.370	26.442	1.00	24.89	C
ATOM	2262	O	VAL	A	271	43.923	33.115	25.980	1.00	21.98	O
ATOM	2263	CB	VAL	A	271	42.901	35.813	26.736	1.00	29.25	C
ATOM	2264	CG1	VAL	A	271	42.256	35.773	25.370	1.00	31.91	C
ATOM	2265	CG2	VAL	A	271	42.421	36.989	27.565	1.00	18.72	C
ATOM	2266	N	PHE	A	272	41.716	32.758	26.019	1.00	26.14	N
ATOM	2267	CA	PHE	A	272	41.752	31.747	24.963	1.00	24.34	C
ATOM	2268	C	PHE	A	272	41.236	32.266	23.623	1.00	28.95	C
ATOM	2269	O	PHE	A	272	40.155	32.826	23.582	1.00	22.01	O
ATOM	2270	CB	PHE	A	272	40.960	30.506	25.391	1.00	20.97	C
ATOM	2271	CG	PHE	A	272	41.764	29.570	26.243	1.00	21.77	C
ATOM	2272	CD1	PHE	A	272	41.940	29.842	27.610	1.00	14.60	C
ATOM	2273	CD2	PHE	A	272	42.504	28.550	25.656	1.00	22.19	C
ATOM	2274	CE1	PHE	A	272	42.763	29.041	28.434	1.00	17.89	C
ATOM	2275	CE2	PHE	A	272	43.336	27.726	26.454	1.00	27.64	C
ATOM	2276	CZ	PHE	A	272	43.478	27.979	27.851	1.00	25.14	C
ATOM	2277	N	ASP	A	273	42.012	32.114	22.542	1.00	29.45	N
ATOM	2278	CA	ASP	A	273	41.557	32.536	21.214	1.00	22.33	C
ATOM	2279	C	ASP	A	273	40.896	31.365	20.493	1.00	25.67	C
ATOM	2280	O	ASP	A	273	41.539	30.570	19.793	1.00	17.81	O
ATOM	2281	CB	ASP	A	273	42.672	33.114	20.343	1.00	21.45	C
ATOM	2282	CG	ASP	A	273	42.131	33.626	18.990	1.00	26.89	C
ATOM	2283	OD1	ASP	A	273	40.975	33.249	18.598	1.00	27.76	O
ATOM	2284	OD2	ASP	A	273	42.838	34.421	18.327	1.00	30.06	O
ATOM	2285	N	ALA	A	274	39.589	31.284	20.649	1.00	15.59	N
ATOM	2286	CA	ALA	A	274	38.932	30.128	20.128	1.00	23.75	C
ATOM	2287	C	ALA	A	274	38.853	30.168	18.653	1.00	32.30	C
ATOM	2288	O	ALA	A	274	38.284	29.256	18.029	1.00	29.37	O
ATOM	2289	CB	ALA	A	274	37.567	29.905	20.777	1.00	18.87	C
ATOM	2290	N	SER	A	275	39.372	31.243	18.081	1.00	21.10	N
ATOM	2291	CA	SER	A	275	39.343	31.288	16.631	1.00	26.90	C
ATOM	2292	C	SER	A	275	40.390	30.300	16.116	1.00	43.37	C
ATOM	2293	O	SER	A	275	40.421	29.949	14.927	1.00	46.32	O
ATOM	2294	CB	SER	A	275	39.547	32.683	16.074	1.00	15.19	C
ATOM	2295	OG	SER	A	275	40.904	33.070	16.078	1.00	28.71	O
ATOM	2296	N	LYS	A	276	41.192	29.780	17.037	1.00	22.98	N
ATOM	2297	CA	LYS	A	276	42.178	28.791	16.638	1.00	23.28	C
ATOM	2298	C	LYS	A	276	41.645	27.405	16.976	1.00	29.73	C
ATOM	2299	O	LYS	A	276	40.992	27.206	18.010	1.00	25.10	O
ATOM	2300	CB	LYS	A	276	43.544	29.051	17.275	1.00	19.19	C
ATOM	2301	CG	LYS	A	276	43.957	30.496	17.218	1.00	32.11	C
ATOM	2302	CD	LYS	A	276	44.062	30.852	15.798	1.00	22.43	C
ATOM	2303	CE	LYS	A	276	44.930	32.067	15.570	1.00	23.18	C
ATOM	2304	NZ	LYS	A	276	45.454	32.117	14.152	1.00	29.42	N

ATOM	2305	N	PRO	A	277	41.892	26.476	16.055	1.00	36.04	N
ATOM	2306	CA	PRO	A	277	41.446	25.087	16.170	1.00	35.93	C
ATOM	2307	C	PRO	A	277	42.022	24.332	17.363	1.00	29.30	C
ATOM	2308	O	PRO	A	277	43.103	24.650	17.885	1.00	30.54	O
ATOM	2309	CB	PRO	A	277	41.975	24.453	14.878	1.00	39.65	C
ATOM	2310	CG	PRO	A	277	43.249	25.261	14.566	1.00	42.90	C
ATOM	2311	CD	PRO	A	277	42.787	26.670	14.892	1.00	37.84	C
ATOM	2312	N	ASP	A	278	41.273	23.339	17.809	1.00	22.35	N
ATOM	2313	CA	ASP	A	278	41.745	22.501	18.903	1.00	22.16	C
ATOM	2314	C	ASP	A	278	42.184	21.189	18.272	1.00	19.66	C
ATOM	2315	O	ASP	A	278	41.905	20.917	17.117	1.00	23.49	O
ATOM	2316	CB	ASP	A	278	40.636	22.241	19.971	1.00	15.09	C
ATOM	2317	CG	ASP	A	278	40.216	23.503	20.702	1.00	22.86	C
ATOM	2318	OD1	ASP	A	278	41.113	24.254	21.096	1.00	25.18	O
ATOM	2319	OD2	ASP	A	278	38.999	23.787	20.812	1.00	39.55	O
ATOM	2320	N	GLY	A	279	42.846	20.355	19.044	1.00	30.65	N
ATOM	2321	CA	GLY	A	279	43.229	19.034	18.546	1.00	33.78	C
ATOM	2322	C	GLY	A	279	42.115	18.099	18.944	1.00	38.10	C
ATOM	2323	O	GLY	A	279	40.963	18.517	19.068	1.00	47.52	O
ATOM	2324	N	THR	A	280	42.419	16.839	19.177	1.00	29.44	N
ATOM	2325	CA	THR	A	280	41.328	15.990	19.587	1.00	26.68	C
ATOM	2326	C	THR	A	280	40.889	16.439	20.972	1.00	23.52	C
ATOM	2327	O	THR	A	280	41.670	17.067	21.713	1.00	23.62	O
ATOM	2328	CB	THR	A	280	41.695	14.492	19.540	1.00	40.78	C
ATOM	2329	OG1	THR	A	280	42.889	14.272	20.296	1.00	25.56	O
ATOM	2330	CG2	THR	A	280	41.893	14.054	18.095	1.00	37.71	C
ATOM	2331	N	PRO	A	281	39.672	16.063	21.346	1.00	25.54	N
ATOM	2332	CA	PRO	A	281	39.129	16.454	22.628	1.00	25.72	C
ATOM	2333	C	PRO	A	281	39.776	15.778	23.800	1.00	26.02	C
ATOM	2334	O	PRO	A	281	39.752	16.314	24.915	1.00	22.68	O
ATOM	2335	CB	PRO	A	281	37.650	15.990	22.559	1.00	28.89	C
ATOM	2336	CG	PRO	A	281	37.417	15.540	21.201	1.00	29.39	C
ATOM	2337	CD	PRO	A	281	38.761	15.138	20.646	1.00	26.82	C
ATOM	2338	N	ARG	A	282	40.281	14.567	23.587	1.00	27.88	N
ATOM	2339	CA	ARG	A	282	40.806	13.817	24.720	1.00	34.08	C
ATOM	2340	C	ARG	A	282	41.977	12.918	24.384	1.00	27.62	C
ATOM	2341	O	ARG	A	282	41.913	12.182	23.425	1.00	23.83	O
ATOM	2342	CB	ARG	A	282	39.676	13.017	25.405	1.00	20.89	C
ATOM	2343	CG	ARG	A	282	40.035	12.467	26.775	1.00	22.81	C
ATOM	2344	CD	ARG	A	282	38.762	11.925	27.442	1.00	26.77	C
ATOM	2345	NE	ARG	A	282	38.963	11.345	28.781	1.00	36.48	N
ATOM	2346	CZ	ARG	A	282	38.518	10.139	29.164	1.00	37.74	C
ATOM	2347	NH1	ARG	A	282	37.813	9.360	28.346	1.00	28.45	N
ATOM	2348	NH2	ARG	A	282	38.754	9.700	30.384	1.00	27.25	N
ATOM	2349	N	LYS	A	283	43.016	12.963	25.223	1.00	28.91	N

ATOM	2350	CA	LYS	A	283	44.217	12.171	25.051	1.00	24.32	C
ATOM	2351	C	LYS	A	283	44.796	11.766	26.404	1.00	29.57	C
ATOM	2352	O	LYS	A	283	45.262	12.626	27.138	1.00	33.16	O
ATOM	2353	CB	LYS	A	283	45.226	13.008	24.287	1.00	21.93	C
ATOM	2354	CG	LYS	A	283	46.111	12.251	23.316	1.00	32.38	C
ATOM	2355	CD	LYS	A	283	46.526	13.171	22.143	1.00	95.77	C
ATOM	2356	CE	LYS	A	283	45.710	12.937	20.836	1.00100.00		C
ATOM	2357	NZ	LYS	A	283	46.418	13.332	19.535	1.00100.00		N
ATOM	2358	N	LEU	A	284	44.747	10.467	26.734	1.00	23.37	N
ATOM	2359	CA	LEU	A	284	45.327	9.905	27.997	1.00	16.08	C
ATOM	2360	C	LEU	A	284	45.463	8.386	28.047	1.00	20.46	C
ATOM	2361	O	LEU	A	284	44.679	7.655	27.446	1.00	25.45	O
ATOM	2362	CB	LEU	A	284	44.641	10.387	29.284	1.00	16.30	C
ATOM	2363	CG	LEU	A	284	43.334	9.700	29.714	1.00	25.97	C
ATOM	2364	CD1	LEU	A	284	42.881	10.089	31.152	1.00	22.11	C
ATOM	2365	CD2	LEU	A	284	42.203	9.953	28.693	1.00	23.92	C
ATOM	2366	N	LEU	A	285	46.453	7.939	28.820	1.00	18.51	N
ATOM	2367	CA	LEU	A	285	46.792	6.527	29.003	1.00	16.77	C
ATOM	2368	C	LEU	A	285	45.880	5.865	30.006	1.00	30.75	C
ATOM	2369	O	LEU	A	285	45.576	6.439	31.058	1.00	22.02	O
ATOM	2370	CB	LEU	A	285	48.229	6.389	29.585	1.00	15.85	C
ATOM	2371	CG	LEU	A	285	49.307	6.970	28.672	1.00	21.51	C
ATOM	2372	CD1	LEU	A	285	50.703	6.705	29.122	1.00	15.15	C
ATOM	2373	CD2	LEU	A	285	49.051	6.368	27.330	1.00	16.94	C
ATOM	2374	N	ASP	A	286	45.565	4.599	29.734	1.00	26.62	N
ATOM	2375	CA	ASP	A	286	44.945	3.726	30.698	1.00	10.90	C
ATOM	2376	C	ASP	A	286	46.128	3.055	31.498	1.00	20.54	C
ATOM	2377	O	ASP	A	286	46.991	2.372	30.938	1.00	23.38	O
ATOM	2378	CB	ASP	A	286	44.073	2.702	29.970	1.00	14.65	C
ATOM	2379	CG	ASP	A	286	43.409	1.699	30.943	1.00	24.60	C
ATOM	2380	OD1	ASP	A	286	43.932	1.437	32.083	1.00	24.60	O
ATOM	2381	OD2	ASP	A	286	42.316	1.231	30.583	1.00	26.03	O
ATOM	2382	N	VAL	A	287	46.230	3.317	32.791	1.00	15.44	N
ATOM	2383	CA	VAL	A	287	47.354	2.816	33.556	1.00	15.58	C
ATOM	2384	C	VAL	A	287	46.973	1.695	34.521	1.00	16.48	C
ATOM	2385	O	VAL	A	287	47.613	1.473	35.572	1.00	16.63	O
ATOM	2386	CB	VAL	A	287	48.101	4.006	34.260	1.00	29.84	C
ATOM	2387	CG1	VAL	A	287	48.534	5.085	33.224	1.00	18.39	C
ATOM	2388	CG2	VAL	A	287	47.173	4.670	35.258	1.00	37.79	C
ATOM	2389	N	THR	A	288	45.904	0.992	34.152	1.00	22.27	N
ATOM	2390	CA	THR	A	288	45.428	-0.152	34.956	1.00	19.34	C
ATOM	2391	C	THR	A	288	46.561	-1.177	35.227	1.00	27.47	C
ATOM	2392	O	THR	A	288	46.778	-1.586	36.365	1.00	24.87	O
ATOM	2393	CB	THR	A	288	44.288	-0.909	34.244	1.00	22.86	C
ATOM	2394	OG1	THR	A	288	43.120	-0.096	34.106	1.00	24.84	O

ATOM	2395	CG2	THR	A	288	43.916	-2.113	35.024	1.00	25.08	C
ATOM	2396	N	ARG	A	289	47.290	-1.585	34.179	1.00	26.08	N
ATOM	2397	CA	ARG	A	289	48.428	-2.506	34.319	1.00	16.92	C
ATOM	2398	C	ARG	A	289	49.405	-2.037	35.408	1.00	22.96	C
ATOM	2399	O	ARG	A	289	49.847	-2.790	36.275	1.00	23.03	O
ATOM	2400	CB	ARG	A	289	49.208	-2.607	32.976	1.00	12.43	C
ATOM	2401	CG	ARG	A	289	48.934	-3.804	32.103	1.00	29.39	C
ATOM	2402	CD	ARG	A	289	50.016	-4.102	31.037	1.00	25.88	C
ATOM	2403	NE	ARG	A	289	49.441	-4.996	30.020	1.00	17.26	N
ATOM	2404	CZ	ARG	A	289	50.053	-5.459	28.930	1.00	38.82	C
ATOM	2405	NH1	ARG	A	289	51.306	-5.153	28.660	1.00	13.51	N
ATOM	2406	NH2	ARG	A	289	49.400	-6.262	28.096	1.00	37.68	N
ATOM	2407	N	LEU	A	290	49.815	-0.786	35.306	1.00	26.60	N
ATOM	2408	CA	LEU	A	290	50.809	-0.254	36.219	1.00	25.42	C
ATOM	2409	C	LEU	A	290	50.324	-0.376	37.656	1.00	24.17	C
ATOM	2410	O	LEU	A	290	51.072	-0.759	38.574	1.00	19.94	O
ATOM	2411	CB	LEU	A	290	51.000	1.219	35.876	1.00	24.66	C
ATOM	2412	CG	LEU	A	290	52.281	2.019	36.066	1.00	24.67	C
ATOM	2413	CD1	LEU	A	290	51.992	3.479	36.504	1.00	29.25	C
ATOM	2414	CD2	LEU	A	290	53.450	1.335	36.788	1.00	15.82	C
ATOM	2415	N	HIS	A	291	49.093	0.075	37.868	1.00	30.10	N
ATOM	2416	CA	HIS	A	291	48.513	0.074	39.212	1.00	34.17	C
ATOM	2417	C	HIS	A	291	48.411	-1.367	39.730	1.00	43.41	C
ATOM	2418	O	HIS	A	291	48.621	-1.654	40.929	1.00	38.81	O
ATOM	2419	CB	HIS	A	291	47.113	0.674	39.143	1.00	28.01	C
ATOM	2420	CG	HIS	A	291	47.097	2.153	38.984	1.00	29.68	C
ATOM	2421	ND1	HIS	A	291	48.242	2.921	39.015	1.00	35.63	N
ATOM	2422	CD2	HIS	A	291	46.068	3.024	38.855	1.00	31.18	C
ATOM	2423	CE1	HIS	A	291	47.926	4.197	38.845	1.00	24.20	C
ATOM	2424	NE2	HIS	A	291	46.612	4.289	38.747	1.00	21.92	N
ATOM	2425	N	GLN	A	292	48.048	-2.260	38.821	1.00	30.71	N
ATOM	2426	CA	GLN	A	292	47.950	-3.654	39.181	1.00	34.82	C
ATOM	2427	C	GLN	A	292	49.287	-4.197	39.622	1.00	36.93	C
ATOM	2428	O	GLN	A	292	49.323	-5.040	40.510	1.00	27.56	O
ATOM	2429	CB	GLN	A	292	47.322	-4.487	38.069	1.00	28.23	C
ATOM	2430	CG	GLN	A	292	45.798	-4.405	38.171	1.00	81.15	C
ATOM	2431	CD	GLN	A	292	45.023	-4.954	36.963	1.00	100.00	C
ATOM	2432	OE1	GLN	A	292	45.597	-5.410	35.951	1.00	99.65	O
ATOM	2433	NE2	GLN	A	292	43.687	-4.895	37.073	1.00	40.86	N
ATOM	2434	N	LEU	A	293	50.375	-3.658	39.058	1.00	31.75	N
ATOM	2435	CA	LEU	A	293	51.750	-4.072	39.383	1.00	22.67	C
ATOM	2436	C	LEU	A	293	52.238	-3.323	40.613	1.00	28.64	C
ATOM	2437	O	LEU	A	293	53.420	-3.377	41.017	1.00	22.27	O
ATOM	2438	CB	LEU	A	293	52.665	-3.769	38.205	1.00	25.57	C
ATOM	2439	CG	LEU	A	293	52.497	-4.703	37.016	1.00	35.11	C

ATOM	2440	CD1	LEU	A	293	53.306	-4.170	35.836	1.00	28.25	C
ATOM	2441	CD2	LEU	A	293	52.965	-6.110	37.439	1.00	47.81	C
ATOM	2442	N	GLY	A	294	51.316	-2.510	41.111	1.00	33.08	N
ATOM	2443	CA	GLY	A	294	51.488	-1.793	42.347	1.00	24.90	C
ATOM	2444	C	GLY	A	294	52.272	-0.512	42.326	1.00	29.31	C
ATOM	2445	O	GLY	A	294	53.070	-0.249	43.223	1.00	25.25	O
ATOM	2446	N	TRP	A	295	52.000	0.347	41.368	1.00	27.83	N
ATOM	2447	CA	TRP	A	295	52.687	1.623	41.385	1.00	19.45	C
ATOM	2448	C	TRP	A	295	51.684	2.731	41.081	1.00	25.79	C
ATOM	2449	O	TRP	A	295	50.765	2.527	40.297	1.00	20.43	O
ATOM	2450	CB	TRP	A	295	53.961	1.614	40.524	1.00	12.85	C
ATOM	2451	CG	TRP	A	295	54.750	2.911	40.618	1.00	23.04	C
ATOM	2452	CD1	TRP	A	295	55.897	3.161	41.368	1.00	23.68	C
ATOM	2453	CD2	TRP	A	295	54.415	4.159	39.979	1.00	20.72	C
ATOM	2454	NE1	TRP	A	295	56.258	4.493	41.244	1.00	18.67	N
ATOM	2455	CE2	TRP	A	295	55.389	5.113	40.373	1.00	20.95	C
ATOM	2456	CE3	TRP	A	295	53.406	4.550	39.102	1.00	21.47	C
ATOM	2457	CZ2	TRP	A	295	55.338	6.439	39.958	1.00	17.58	C
ATOM	2458	CZ3	TRP	A	295	53.403	5.873	38.632	1.00	21.57	C
ATOM	2459	CH2	TRP	A	295	54.368	6.787	39.058	1.00	19.45	C
ATOM	2460	N	TYR	A	296	51.709	3.797	41.884	1.00	25.17	N
ATOM	2461	CA	TYR	A	296	50.720	4.883	41.731	1.00	24.90	C
ATOM	2462	C	TYR	A	296	51.517	6.178	41.857	1.00	30.85	C
ATOM	2463	O	TYR	A	296	52.363	6.272	42.745	1.00	21.27	O
ATOM	2464	CB	TYR	A	296	49.654	4.813	42.840	1.00	25.18	C
ATOM	2465	CG	TYR	A	296	48.685	3.651	42.744	1.00	23.04	C
ATOM	2466	CD1	TYR	A	296	49.078	2.343	43.088	1.00	31.62	C
ATOM	2467	CD2	TYR	A	296	47.380	3.853	42.289	1.00	26.02	C
ATOM	2468	CE1	TYR	A	296	48.203	1.268	42.935	1.00	24.42	C
ATOM	2469	CE2	TYR	A	296	46.493	2.770	42.127	1.00	24.81	C
ATOM	2470	CZ	TYR	A	296	46.902	1.483	42.464	1.00	39.41	C
ATOM	2471	OH	TYR	A	296	45.984	0.434	42.337	1.00	66.19	O
ATOM	2472	N	HIS	A	297	51.324	7.123	40.924	1.00	20.95	N
ATOM	2473	CA	HIS	A	297	52.130	8.343	40.938	1.00	26.86	C
ATOM	2474	C	HIS	A	297	51.947	9.175	42.210	1.00	35.01	C
ATOM	2475	O	HIS	A	297	50.885	9.132	42.874	1.00	26.92	O
ATOM	2476	CB	HIS	A	297	51.819	9.192	39.733	1.00	25.77	C
ATOM	2477	CG	HIS	A	297	50.489	9.842	39.803	1.00	31.16	C
ATOM	2478	ND1	HIS	A	297	49.314	9.145	39.633	1.00	34.21	N
ATOM	2479	CD2	HIS	A	297	50.135	11.094	40.167	1.00	25.83	C
ATOM	2480	CE1	HIS	A	297	48.290	9.972	39.776	1.00	24.14	C
ATOM	2481	NE2	HIS	A	297	48.761	11.164	40.087	1.00	23.35	N
ATOM	2482	N	GLU	A	298	52.983	9.926	42.554	1.00	24.98	N
ATOM	2483	CA	GLU	A	298	52.957	10.683	43.798	1.00	27.65	C
ATOM	2484	C	GLU	A	298	52.831	12.187	43.741	1.00	36.86	C

ATOM	2485	O	GLU	A	298	52.433	12.792	44.718	1.00	43.61	O
ATOM	2486	CB	GLU	A	298	54.153	10.319	44.686	1.00	22.02	C
ATOM	2487	CG	GLU	A	298	54.004	8.943	45.285	1.00	36.42	C
ATOM	2488	CD	GLU	A	298	54.999	8.664	46.406	1.00	100.00	C
ATOM	2489	OE1	GLU	A	298	56.223	8.561	46.152	1.00	44.79	O
ATOM	2490	OE2	GLU	A	298	54.526	8.470	47.547	1.00	100.00	O
ATOM	2491	N	ILE	A	299	53.232	12.800	42.639	1.00	23.49	N
ATOM	2492	CA	ILE	A	299	53.268	14.244	42.562	1.00	13.25	C
ATOM	2493	C	ILE	A	299	52.016	14.848	41.906	1.00	27.05	C
ATOM	2494	O	ILE	A	299	51.681	14.530	40.757	1.00	26.73	O
ATOM	2495	CB	ILE	A	299	54.586	14.711	41.862	1.00	15.93	C
ATOM	2496	CG1	ILE	A	299	55.836	14.183	42.606	1.00	23.83	C
ATOM	2497	CG2	ILE	A	299	54.596	16.213	41.541	1.00	17.37	C
ATOM	2498	CD1	ILE	A	299	57.232	14.221	41.787	1.00	21.32	C
ATOM	2499	N	SER	A	300	51.323	15.716	42.648	1.00	18.55	N
ATOM	2500	CA	SER	A	300	50.177	16.449	42.091	1.00	19.58	C
ATOM	2501	C	SER	A	300	50.714	17.415	41.042	1.00	17.29	C
ATOM	2502	O	SER	A	300	51.824	17.941	41.178	1.00	21.06	O
ATOM	2503	CB	SER	A	300	49.542	17.307	43.181	1.00	16.78	C
ATOM	2504	OG	SER	A	300	50.548	17.969	43.923	1.00	75.80	O
ATOM	2505	N	LEU	A	301	49.870	17.755	40.075	1.00	16.13	N
ATOM	2506	CA	LEU	A	301	50.246	18.675	39.014	1.00	17.70	C
ATOM	2507	C	LEU	A	301	50.689	19.964	39.646	1.00	20.11	C
ATOM	2508	O	LEU	A	301	51.714	20.568	39.303	1.00	20.46	O
ATOM	2509	CB	LEU	A	301	48.990	18.981	38.197	1.00	17.92	C
ATOM	2510	CG	LEU	A	301	49.182	20.030	37.112	1.00	25.15	C
ATOM	2511	CD1	LEU	A	301	50.233	19.552	36.086	1.00	18.82	C
ATOM	2512	CD2	LEU	A	301	47.854	20.177	36.436	1.00	25.88	C
ATOM	2513	N	GLU	A	302	49.845	20.398	40.554	1.00	27.01	N
ATOM	2514	CA	GLU	A	302	50.053	21.636	41.280	1.00	37.72	C
ATOM	2515	C	GLU	A	302	51.410	21.618	41.996	1.00	29.99	C
ATOM	2516	O	GLU	A	302	52.245	22.514	41.798	1.00	27.15	O
ATOM	2517	CB	GLU	A	302	48.899	21.841	42.275	1.00	43.10	C
ATOM	2518	CG	GLU	A	302	49.061	23.061	43.174	1.00	90.85	C
ATOM	2519	CD	GLU	A	302	48.451	24.324	42.580	1.00	100.00	C
ATOM	2520	OE1	GLU	A	302	47.566	24.209	41.706	1.00	100.00	O
ATOM	2521	OE2	GLU	A	302	48.808	25.432	43.036	1.00	64.50	O
ATOM	2522	N	ALA	A	303	51.646	20.591	42.801	1.00	8.72	N
ATOM	2523	CA	ALA	A	303	52.937	20.455	43.459	1.00	15.03	C
ATOM	2524	C	ALA	A	303	54.102	20.355	42.450	1.00	19.85	C
ATOM	2525	O	ALA	A	303	55.104	21.090	42.553	1.00	22.24	O
ATOM	2526	CB	ALA	A	303	52.938	19.258	44.410	1.00	18.97	C
ATOM	2527	N	GLY	A	304	53.953	19.472	41.467	1.00	13.05	N
ATOM	2528	CA	GLY	A	304	54.970	19.321	40.448	1.00	8.94	C
ATOM	2529	C	GLY	A	304	55.239	20.621	39.695	1.00	20.31	C

ATOM	2530	O	GLY	A	304	56.394	20.900	39.322	1.00	14.30	O
ATOM	2531	N	LEU	A	305	54.191	21.383	39.361	1.00	10.76	N
ATOM	2532	CA	LEU	A	305	54.483	22.622	38.611	1.00	20.29	C
ATOM	2533	C	LEU	A	305	55.281	23.669	39.456	1.00	28.92	C
ATOM	2534	O	LEU	A	305	56.194	24.385	38.974	1.00	17.69	O
ATOM	2535	CB	LEU	A	305	53.202	23.245	38.033	1.00	24.03	C
ATOM	2536	CG	LEU	A	305	52.357	22.647	36.880	1.00	27.66	C
ATOM	2537	CD1	LEU	A	305	50.975	23.384	36.789	1.00	13.44	C
ATOM	2538	CD2	LEU	A	305	53.079	22.724	35.543	1.00	18.39	C
ATOM	2539	N	ALA	A	306	54.904	23.757	40.724	1.00	19.94	N
ATOM	2540	CA	ALA	A	306	55.544	24.660	41.655	1.00	24.79	C
ATOM	2541	C	ALA	A	306	57.035	24.380	41.743	1.00	27.51	C
ATOM	2542	O	ALA	A	306	57.852	25.280	41.662	1.00	29.68	O
ATOM	2543	CB	ALA	A	306	54.937	24.471	43.002	1.00	17.87	C
ATOM	2544	N	SER	A	307	57.378	23.137	42.011	1.00	18.46	N
ATOM	2545	CA	SER	A	307	58.793	22.756	42.162	1.00	16.31	C
ATOM	2546	C	SER	A	307	59.547	22.885	40.832	1.00	22.66	C
ATOM	2547	O	SER	A	307	60.742	23.212	40.786	1.00	28.47	O
ATOM	2548	CB	SER	A	307	58.851	21.304	42.622	1.00	20.47	C
ATOM	2549	OG	SER	A	307	58.517	20.454	41.526	1.00	29.03	O
ATOM	2550	N	THR	A	308	58.849	22.631	39.735	1.00	27.31	N
ATOM	2551	CA	THR	A	308	59.458	22.738	38.413	1.00	22.89	C
ATOM	2552	C	THR	A	308	59.757	24.216	38.107	1.00	26.06	C
ATOM	2553	O	THR	A	308	60.819	24.546	37.591	1.00	29.89	O
ATOM	2554	CB	THR	A	308	58.536	22.115	37.318	1.00	18.72	C
ATOM	2555	OG1	THR	A	308	58.356	20.714	37.545	1.00	20.17	O
ATOM	2556	CG2	THR	A	308	59.094	22.330	35.923	1.00	12.37	C
ATOM	2557	N	TYR	A	309	58.846	25.118	38.453	1.00	28.20	N
ATOM	2558	CA	TYR	A	309	59.110	26.549	38.241	1.00	31.09	C
ATOM	2559	C	TYR	A	309	60.383	27.059	39.045	1.00	16.31	C
ATOM	2560	O	TYR	A	309	61.179	27.858	38.577	1.00	16.91	O
ATOM	2561	CB	TYR	A	309	57.819	27.373	38.533	1.00	31.19	C
ATOM	2562	CG	TYR	A	309	57.944	28.895	38.392	1.00	14.57	C
ATOM	2563	CD1	TYR	A	309	58.397	29.457	37.224	1.00	17.51	C
ATOM	2564	CD2	TYR	A	309	57.575	29.757	39.442	1.00	24.99	C
ATOM	2565	CE1	TYR	A	309	58.527	30.801	37.100	1.00	18.41	C
ATOM	2566	CE2	TYR	A	309	57.744	31.129	39.351	1.00	19.04	C
ATOM	2567	CZ	TYR	A	309	58.212	31.641	38.164	1.00	29.13	C
ATOM	2568	OH	TYR	A	309	58.300	33.004	37.966	1.00	28.22	O
ATOM	2569	N	GLN	A	310	60.560	26.579	40.260	1.00	15.41	N
ATOM	2570	CA	GLN	A	310	61.705	26.964	41.087	1.00	22.35	C
ATOM	2571	C	GLN	A	310	63.001	26.492	40.446	1.00	31.46	C
ATOM	2572	O	GLN	A	310	64.009	27.191	40.442	1.00	33.42	O
ATOM	2573	CB	GLN	A	310	61.587	26.335	42.482	1.00	17.67	C
ATOM	2574	CG	GLN	A	310	62.579	26.921	43.461	1.00	57.58	C

ATOM	2575	CD	GLN	A	310	62.287	28.370	43.782	1.00	65.14	C
ATOM	2576	OE1	GLN	A	310	61.134	28.754	44.000	1.00	41.94	O
ATOM	2577	NE2	GLN	A	310	63.330	29.194	43.801	1.00	99.09	N
ATOM	2578	N	TRP	A	311	62.957	25.321	39.830	1.00	28.76	N
ATOM	2579	CA	TRP	A	311	64.146	24.822	39.163	1.00	26.29	C
ATOM	2580	C	TRP	A	311	64.474	25.769	38.040	1.00	17.91	C
ATOM	2581	O	TRP	A	311	65.599	26.193	37.880	1.00	22.89	O
ATOM	2582	CB	TRP	A	311	63.938	23.383	38.643	1.00	27.53	C
ATOM	2583	CG	TRP	A	311	65.176	22.784	38.119	1.00	17.82	C
ATOM	2584	CD1	TRP	A	311	66.132	22.090	38.826	1.00	20.21	C
ATOM	2585	CD2	TRP	A	311	65.652	22.881	36.784	1.00	17.99	C
ATOM	2586	NE1	TRP	A	311	67.197	21.776	37.992	1.00	20.39	N
ATOM	2587	CE2	TRP	A	311	66.933	22.284	36.746	1.00	19.57	C
ATOM	2588	CE3	TRP	A	311	65.141	23.461	35.621	1.00	20.26	C
ATOM	2589	CZ2	TRP	A	311	67.686	22.236	35.599	1.00	14.25	C
ATOM	2590	CZ3	TRP	A	311	65.901	23.446	34.501	1.00	18.59	C
ATOM	2591	CH2	TRP	A	311	67.169	22.831	34.494	1.00	16.86	C
ATOM	2592	N	PHE	A	312	63.469	26.109	37.256	1.00	17.47	N
ATOM	2593	CA	PHE	A	312	63.665	27.064	36.179	1.00	20.14	C
ATOM	2594	C	PHE	A	312	64.224	28.371	36.733	1.00	18.33	C
ATOM	2595	O	PHE	A	312	65.080	29.024	36.104	1.00	24.76	O
ATOM	2596	CB	PHE	A	312	62.328	27.318	35.458	1.00	29.51	C
ATOM	2597	CG	PHE	A	312	62.328	28.544	34.603	1.00	28.52	C
ATOM	2598	CD1	PHE	A	312	62.883	28.508	33.338	1.00	30.53	C
ATOM	2599	CD2	PHE	A	312	61.825	29.758	35.104	1.00	29.31	C
ATOM	2600	CE1	PHE	A	312	62.936	29.660	32.554	1.00	34.73	C
ATOM	2601	CE2	PHE	A	312	61.900	30.904	34.362	1.00	38.40	C
ATOM	2602	CZ	PHE	A	312	62.432	30.860	33.063	1.00	40.73	C
ATOM	2603	N	LEU	A	313	63.697	28.787	37.876	1.00	22.46	N
ATOM	2604	CA	LEU	A	313	64.170	30.025	38.516	1.00	28.47	C
ATOM	2605	C	LEU	A	313	65.627	29.827	38.898	1.00	37.53	C
ATOM	2606	O	LEU	A	313	66.452	30.693	38.629	1.00	34.20	O
ATOM	2607	CB	LEU	A	313	63.375	30.410	39.783	1.00	20.44	C
ATOM	2608	CG	LEU	A	313	61.955	30.897	39.555	1.00	16.29	C
ATOM	2609	CD1	LEU	A	313	61.499	31.399	40.871	1.00	15.94	C
ATOM	2610	CD2	LEU	A	313	61.959	31.961	38.524	1.00	14.44	C
ATOM	2611	N	GLU	A	314	65.953	28.685	39.508	1.00	30.70	N
ATOM	2612	CA	GLU	A	314	67.353	28.432	39.875	1.00	24.15	C
ATOM	2613	C	GLU	A	314	68.291	28.149	38.703	1.00	36.34	C
ATOM	2614	O	GLU	A	314	69.485	28.047	38.890	1.00	43.10	O
ATOM	2615	CB	GLU	A	314	67.459	27.366	40.947	1.00	19.90	C
ATOM	2616	CG	GLU	A	314	66.634	27.754	42.141	1.00	27.37	C
ATOM	2617	CD	GLU	A	314	66.450	26.666	43.182	1.00	31.09	C
ATOM	2618	OE1	GLU	A	314	67.157	25.648	43.085	1.00	59.60	O
ATOM	2619	OE2	GLU	A	314	65.634	26.872	44.125	1.00	46.20	O

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ATOM   2620  N   ASN A 315      67.778  28.114  37.479  1.00 40.17           N
ATOM   2621  CA  ASN A 315      68.637  27.802  36.343  1.00 37.76           C
ATOM   2622  C   ASN A 315      68.383  28.578  35.112  1.00 43.75           C
ATOM   2623  O   ASN A 315      68.591  28.001  34.047  1.00 39.15           O
ATOM   2624  CB  ASN A 315      68.425  26.360  35.884  1.00 33.74           C
ATOM   2625  CG  ASN A 315      69.028  25.383  36.801  1.00 53.18           C
ATOM   2626  OD1 ASN A 315      68.456  25.087  37.835  1.00 49.13           O
ATOM   2627  ND2 ASN A 315      70.239  24.926  36.479  1.00 97.72           N
ATOM   2628  N   GLN A 316      67.852  29.803  35.197  1.00 49.87           N
ATOM   2629  CA  GLN A 316      67.627  30.550  33.957  1.00 77.90           C
ATOM   2630  C   GLN A 316      68.797  31.448  33.525  1.00100.00           C
ATOM   2631  O   GLN A 316      69.272  31.387  32.375  1.00 51.33           O
ATOM   2632  CB  GLN A 316      66.280  31.276  33.902  1.00 75.89           C
ATOM   2633  CG  GLN A 316      65.683  31.589  35.231  1.00 80.97           C
ATOM   2634  CD  GLN A 316      65.233  33.036  35.350  1.00 54.58           C
ATOM   2635  OE1 GLN A 316      64.881  33.699  34.367  1.00 46.46           O
ATOM   2636  NE2 GLN A 316      65.257  33.538  36.566  1.00 33.46           N
TER    2637      GLN A 316
CONECT  110  111
CONECT  111  110  112
CONECT  112  111  113  114
CONECT  113  112  118
CONECT  114  112  115  116
CONECT  115  114
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CONECT  128  123  126
CONECT  129  117  130  131  132
CONECT  130  129
CONECT  131  129
CONECT  132  129
MASTER      208    0    1   13   10    0    3    6 2636    1   22   25
END
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While various embodiments of the present invention have been described in detail, it is apparent that modifications and adaptations of those embodiments will occur to those skilled in the art. However, it is to be expressly understood that such modifications and adaptations are within the spirit and scope of the present invention, as set forth in the following claims.

What is claimed:

1. A method for producing ascorbic acid or esters thereof in a microorganism, comprising culturing a microorganism having a genetic modification to increase the action of an enzyme selected from the group consisting of hexokinase, glucose phosphate isomerase, phosphomannose isomerase, phosphomannomutase, GDP-D-mannose pyrophosphorylase, GDP-D-mannose:GDP-L-galactose epimerase, GDP-L-galactose phosphorylase, L-galactose-1-P-phosphatase, L-galactose dehydrogenase, and L-galactono-γ-lactone dehydrogenase; and recovering said ascorbic acid or esters thereof.

2. A method, as claimed in Claim 1, wherein said genetic modification is a genetic modification to increase the action of an enzyme selected from the group consisting of GDP-D-mannose:GDP-L-galactose epimerase, GDP-L-galactose phosphorylase, L-galactose-1-P-phosphatase, L-galactose dehydrogenase, and L-galactono-γ-lactone dehydrogenase.

3. A method, as claimed in Claim 1, wherein said genetic modification is a genetic modification to increase the action of an epimerase that catalyzes conversion of GDP-D-mannose to GDP-L-galactose.

4. A method, as claimed in Claim 3, wherein said genetic modification is a genetic modification to increase the action of GDP-D-mannose:GDP-L-galactose epimerase.

5. The method of Claim 3, wherein said genetic modification comprises transformation of said microorganism with a recombinant nucleic acid molecule that expresses said epimerase.

6. The method of Claim 5, wherein said epimerase has a tertiary structure that substantially conforms to the tertiary structure of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws.

7. The method of Claim 5, wherein said epimerase has a structure having an average root mean square deviation of less than about 2.5 Å over at least about 25% of Cα positions of the tertiary structure of a GDP-4-keto-6-deoxy-D-mannose epimerase/ reductase represented by atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws.

8. The method of Claim 5, wherein said epimerase has a tertiary structure having an average root mean square deviation of less than about 1 Å over at least about 25% of Cα positions of the tertiary structure of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws.

9. The method of Claim 5, wherein said epimerase comprises a substrate binding site having a tertiary structure that substantially conforms to the tertiary structure of the substrate binding site of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws.

10. The method of Claim 9, wherein said substrate binding site has a tertiary structure with an average root mean square deviation of less than about 2.5 Å over at least about 25% of Cα positions of the tertiary structure of a substrate binding site of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws.

11. The method of Claim 5, wherein said epimerase comprises a catalytic site having a tertiary structure that substantially conforms to the tertiary structure of the catalytic site of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws.

12. The method of Claim 11, wherein said catalytic site has a tertiary structure with an average root mean square deviation of less than about 2.5 Å over at least about 25% of Cα positions of the tertiary structure of a catalytic site of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws.

13. The method of Claim 11, wherein said catalytic site comprises the amino acid residues serine, tyrosine and lysine.

14. The method of Claim 13, wherein tertiary structure positions of said amino acid residues serine, tyrosine and lysine substantially conform to tertiary structure positions of residues Ser107, Tyr136 and Lys140, respectively, as represented by atomic coordinates in Brookhaven Protein Data Bank Accession Code 1bws.

15. The method of Claim 5, wherein said epimerase binds NADPH.

16. The method of Claim 5, wherein said epimerase comprises an amino acid sequence that aligns with SEQ ID NO:11 using a CLUSTAL alignment program, wherein amino acid residues in said amino acid sequence align with 100% identity with at least about 50% of non-Xaa residues in SEQ ID NO:11.

17. The method of Claim 5, wherein said epimerase comprises an amino acid sequence that aligns with SEQ ID NO:11 using a CLUSTAL alignment program, wherein amino acid residues in said amino acid sequence align with 100% identity with at least about 75% of non-Xaa residues in SEQ ID NO:11.

18. The method of Claim 5, wherein said epimerase comprises an amino acid sequence that aligns with SEQ ID NO:11 using a CLUSTAL alignment program, wherein amino acid residues in said amino acid sequence align with 100% identity with at least about 90% of non-Xaa residues in SEQ ID NO:11.

19. The method of Claim 5, wherein said epimerase comprises an amino acid sequence having at least 4 contiguous amino acid residues that are 100% identical to at least 4 contiguous amino acid residues of an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8 and SEQ ID NO:10.

20. The method of Claim 5, wherein said recombinant nucleic acid molecule comprises a nucleic acid sequence comprising at least about 12 contiguous nucleotides having 100% identity with at least about 12 contiguous nucleotides of a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7 and SEQ ID NO:9.

21. The method of Claim 5, wherein said epimerase comprises an amino acid sequence having a motif: Gly-Xaa-Xaa-Gly-Xaa-Xaa-Gly.

22. The method of Claim 5, wherein said recombinant nucleic acid molecule comprises a nucleic acid sequence that is at least about 15% identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7 and SEQ ID NO:9, as determined using a Lipman-Pearson method with Lipman-Pearson standard default parameters.

23. The method of Claim 5, wherein said recombinant nucleic acid molecule comprises a nucleic acid sequence that is at least about 20% identical to a nucleic acid

sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7 and SEQ ID NO:9, as determined using a Lipman-Pearson method with Lipman-Pearson standard default parameters.

24. The method of Claim 5, wherein said recombinant nucleic acid molecule comprises a nucleic acid sequence that is at least about 25% identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7 and SEQ ID NO:9, as determined using a Lipman-Pearson method with Lipman-Pearson standard default parameters.

25. The method of Claim 5, wherein said recombinant nucleic acid molecule comprises a nucleic acid sequence that hybridizes under stringent hybridization conditions to a nucleic acid sequence encoding a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase.

26. The method of Claim 25, wherein said nucleic acid sequence encoding said GDP-4-keto-6-deoxy-D-mannose epimerase/reductase is selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3 and SEQ ID NO:5.

27. The method of Claim 25, wherein said GDP-4-keto-6-deoxy-D-mannose epimerase/reductase comprises an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4 and SEQ ID NO:6.

28. A method, as claimed in Claim 1, wherein said microorganism is selected from the group consisting of bacteria, fungi and microalgae.

29. A method, as claimed in Claim 1, wherein said microorganism is acid-tolerant.

30. A method, as claimed in Claim 1, wherein said microorganism is a bacterium.

31. A method, as claimed in Claim 30, wherein said bacterium is selected from the group consisting of *Azotobacter* and *Pseudomonas*.

32. A method, as claimed in Claim 1, wherein said microorganism is a fungus.

33. A method, as claimed in Claim 32, wherein said microorganism is a yeast.

34. A method, as claimed in Claim 33, wherein said yeast is selected from the group consisting of *Saccharomyces* yeast.

35. A method, as claimed in Claim 1, wherein said microorganism is a microalga.

36. A method, as claimed in Claim 35, wherein said microalga is selected from the group consisting of microalgae of the genera *Prototheca* and *Chlorella*.

37. A method, as claimed in Claim 36, wherein said microalga is selected from the genus *Prototheca*.

38. A method, as claimed in Claim 1, wherein said microorganism further comprises a genetic modification to decrease the action of an enzyme having GDP-D-mannose as a substrate, other than GDP-D-mannose:GDP-L-galactose epimerase.

39. A method, as claimed in Claim 38, wherein said genetic modification to decrease the action of an enzyme having GDP-D-mannose as a substrate, other than GDP-D-mannose:GDP-L-galactose epimerase is a genetic modification to decrease the action of GDP-D-mannose-dehydrogenase.

40. A method, as claimed in Claim 1, wherein said microorganism is acid-tolerant and said step of culturing is conducted at a pH of less than about 6.0.

41. A method, as claimed in Claim 1, wherein said microorganism is acid-tolerant and said step of culturing is conducted at a pH of less than about 5.5.

42. A method, as claimed in Claim 1, wherein said microorganism is acid-tolerant and said step of culturing is conducted at a pH of less than about 5.0.

43. A method, as claimed in Claim 1, wherein said step of culturing is conducted in a fermentation medium that is magnesium (Mg) limited.

44. A method, as claimed in Claim 1, wherein said step of culturing is conducted in a fermentation medium that is Mg limited during a cell growth phase.

45. A method, as claimed in Claim 1, wherein said step of culturing is conducted in a fermentation medium that comprises less than about 0.5 g/L of Mg during a cell growth phase.

46. A method, as claimed in Claim 1, wherein said step of culturing is conducted in a fermentation medium that comprises less than about 0.2 g/L of Mg during a cell growth phase.

47. A method, as claimed in Claim 1, wherein said step of culturing is conducted in a fermentation medium that comprises less than about 0.1 g/L of Mg during a cell growth phase.

48. A method, as claimed in Claim 1, wherein said step of culturing is conducted in a fermentation medium that comprises a carbon source other than D-mannose.

49. A method, as claimed in Claim 1, wherein said step of culturing is conducted in a fermentation medium that comprises glucose as a carbon source.

50. A microorganism for producing ascorbic acid or esters thereof, wherein said microorganism has a genetic modification to increase the action of an enzyme selected from the group consisting of hexokinase, glucose phosphate isomerase, phosphomannose isomerase, phosphomannomutase, GDP-D-mannose pyrophosphorylase, GDP-D-mannose:GDP-L-galactose epimerase, GDP-L-galactose phosphorylase, L-galactose-1-P-phosphatase, L-galactose dehydrogenase, and L-galactono-γ-lactone dehydrogenase.

51. A microorganism, as claimed in Claim 50, wherein said genetic modification is a genetic modification to increase the action of an enzyme selected from the group consisting of GDP-D-mannose:GDP-L-galactose epimerase, GDP-L-galactose phosphorylase, L-galactose-1-P-phosphatase, L-galactose dehydrogenase, and L-galactono-γ-lactone dehydrogenase.

52. A microorganism, as claimed in Claim 50, wherein said genetic modification is a genetic modification to increase the action of GDP-D-mannose:GDP-L-galactose epimerase.

53. A microorganism, as claimed in Claim 50, wherein said microorganism has been genetically modified to express a recombinant nucleic acid molecule encoding an epimerase that catalyzes conversion of GDP-D-mannose to GDP-L-galactose, wherein said epimerase has a tertiary structure having an average root mean square deviation of less than about 2.5 Å over at least about 25% of Cα positions of the tertiary structure of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws.

54. A microorganism, as claimed in Claim 50, wherein said microorganism is selected from the group consisting of bacteria, fungi and microalgae.

55. A microorganism, as claimed in Claim 50, wherein said microorganism is a bacterium.

56. A microorganism, as claimed in Claim 55, wherein said bacterium is selected from the group consisting of *Azotobacter* and *Pseudomonas*.

57. A microorganism, as claimed in Claim 50, wherein said microorganism is a fungus.

58. A microorganism, as claimed in Claim 57, wherein said microorganism is a yeast.

59. A microorganism, as claimed in Claim 58, wherein said yeast is selected from the group consisting of *Saccharomyces* yeast.

60. A plant for producing ascorbic acid or esters thereof, wherein said plant has a genetic modification to increase the action of an enzyme selected from the group consisting of hexokinase, glucose phosphate isomerase, phosphomannose isomerase, phosphomannomutase, GDP-D-mannose pyrophosphorylase, GDP-D-mannose:GDP-L-galactose epimerase, GDP-L-galactose phosphorylase, L-galactose-1-P-phosphatase, L-galactose dehydrogenase, and L-galactono-γ-lactone dehydrogenase.

61. A plant, as claimed in Claim 60, wherein said genetic modification is a genetic modification to increase the action of an enzyme selected from the group consisting of GDP-D-mannose:GDP-L-galactose epimerase, GDP-L-galactose phosphorylase, L-galactose-1-P-phosphatase, L-galactose dehydrogenase, and L-galactono-γ-lactone dehydrogenase.

62. A plant, as claimed in Claim 60, wherein said genetic modification is a genetic modification to increase the action of GDP-D-mannose:GDP-L-galactose epimerase.

63. A plant, as claimed in Claim 60, wherein said plant has been genetically modified to express a recombinant nucleic acid molecule encoding an epimerase that catalyzes conversion of GDP-D-mannose to GDP-L-galactose, wherein said epimerase has a tertiary structure having an average root mean square deviation of less than about 2.5 Å over at least about 25% of Cα positions of the tertiary structure of a GDP-4-keto-6-

deoxy-D-mannose epimerase/reductase represented by atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws.

64. A plant, as claimed in Claim 60, wherein said plant further comprises a genetic modification to decrease the action of an enzyme having GDP-D-mannose as a substrate other than GDP-D-mannose:GDP-L-galactose epimerase.

65. A plant, as claimed in Claim 60, wherein said genetic modification to decrease the action of an enzyme having GDP-D-mannose as a substrate other than GDP-D-mannose:GDP-L-galactose epimerase is a genetic modification to decrease the action of GDP-D-mannose-dehydrogenase.

66. A plant, as claimed in Claim 60, wherein said plant is a microalga.

67. A plant, as claimed in Claim 66, wherein said plant is selected from the group consisting of microalgae of the genera *Prototheca* and *Chlorella*.

68. A plant, as claimed in Claim 66, wherein said microalga is selected from the genus *Prototheca*.

69. A plant, as claimed in Claim 60, wherein said plant is a higher plant.

70. A plant, as claimed in Claim 60, wherein said plant is a consumable higher plant.

71. A microorganism for producing ascorbic acid or esters thereof, wherein said microorganism has been genetically modified to express a recombinant nucleic acid molecule encoding an epimerase that catalyzes conversion of GDP-D-mannose to GDP-L-galactose, wherein said epimerase comprises an amino acid sequence that aligns with SEQ ID NO:11 using a CLUSTAL alignment program, wherein amino acid residues in said amino acid sequence align with 100% identity with at least about 50% of non-Xaa residues in SEQ ID NO:11.

72. A plant for producing ascorbic acid or esters thereof, wherein said plant has been genetically modified to express a recombinant nucleic acid molecule encoding an epimerase that catalyzes conversion of GDP-D-mannose to GDP-L-galactose, wherein said epimerase comprises an amino acid sequence that aligns with SEQ ID NO:11 using a CLUSTAL alignment program, wherein amino acid residues in said amino acid sequence align with 100% identity with at least about 50% of non-Xaa residues in SEQ ID NO:11.

Proposed Pathway from Glucose to L-Ascorbic Acid through GDP-D-Mannose

Glucose to GDP-mannose

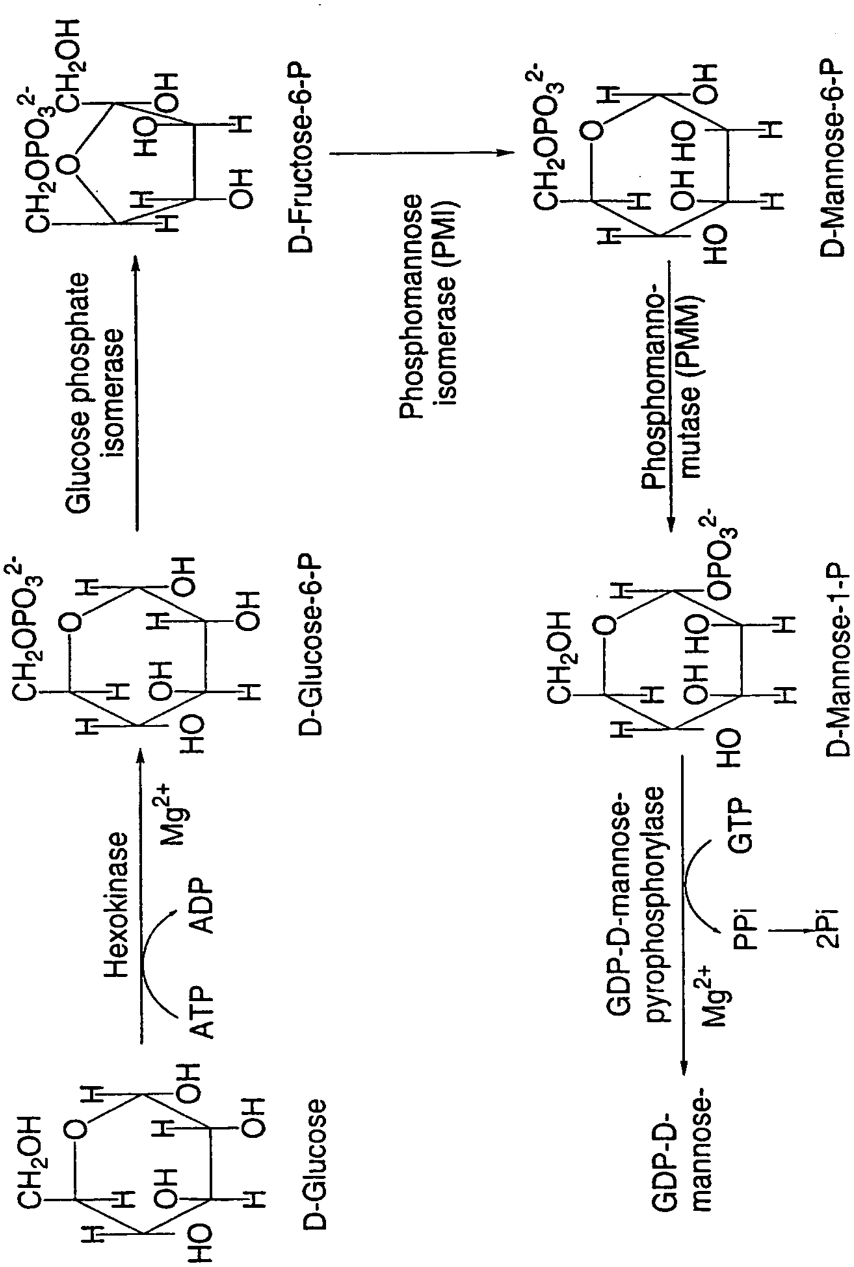

FIG. 1A

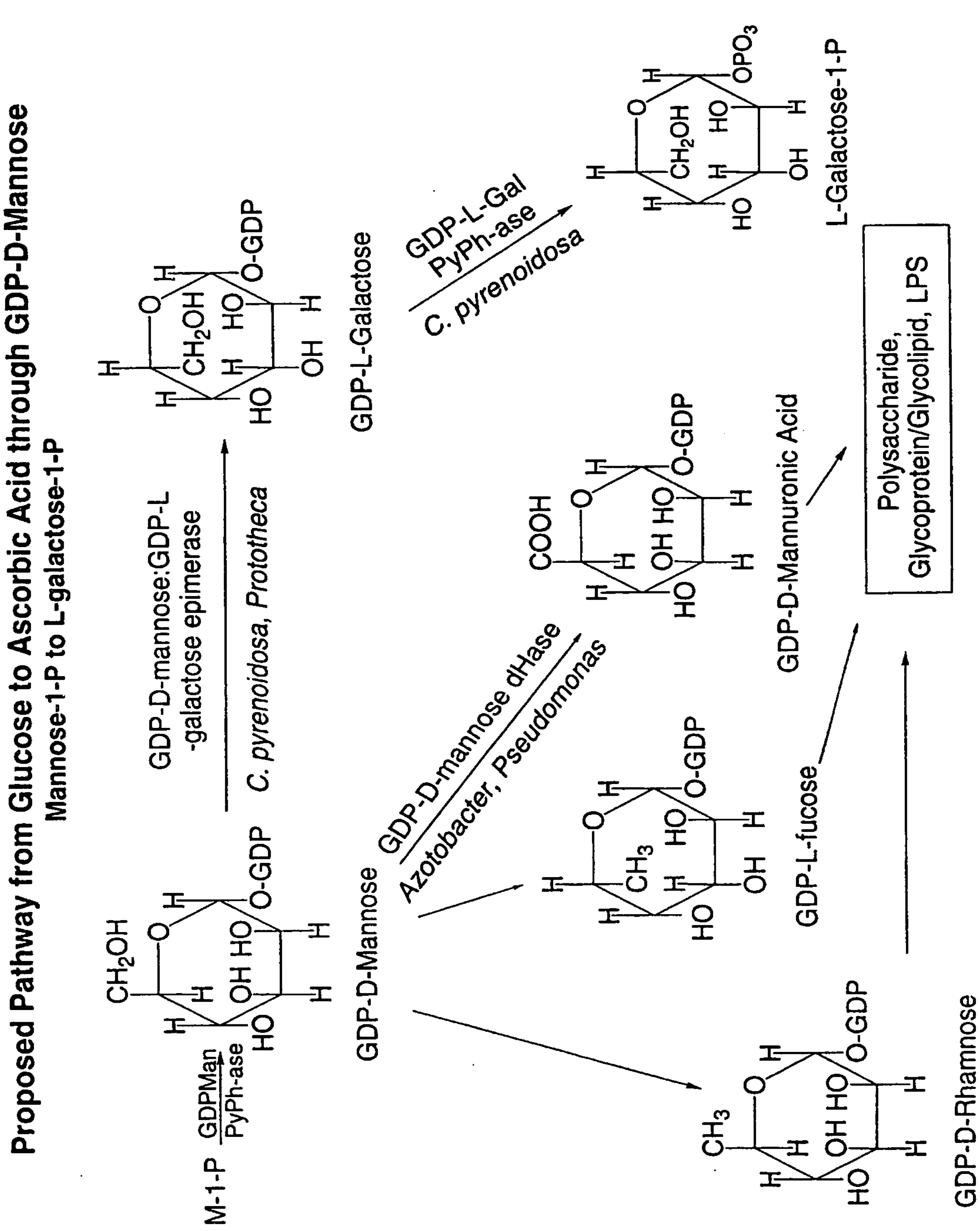
Proposed Pathway from Glucose to Ascorbic Acid through GDP-D-Mannose
Mannose-1-P to L-galactose-1-P
M-1-P
GDPMan PyPh-ase
GDP-D-Mannose
GDP-D-mannose:GDP-L -galactose epimerase
C. pyrenoidosa, Prototheca
GDP-L-Galactose
GDP-L-Gal PyPh-ase
C. pyrenoidosa
L-Galactose-1-P
GDP-D-mannose dHase
Azotobacter, Pseudomonas
GDP-D-Mannuronic Acid
GDP-L-fucose
GDP-D-Rhamnose
Polysaccharide, Glycoprotein/Glycolipid, LPS

FIG. 1B

Proposed Pathway from Glucose to Ascorbic Acid through GDP-D-Mannose

GDP-L-galactose-1-P to L-Ascorbic Acid

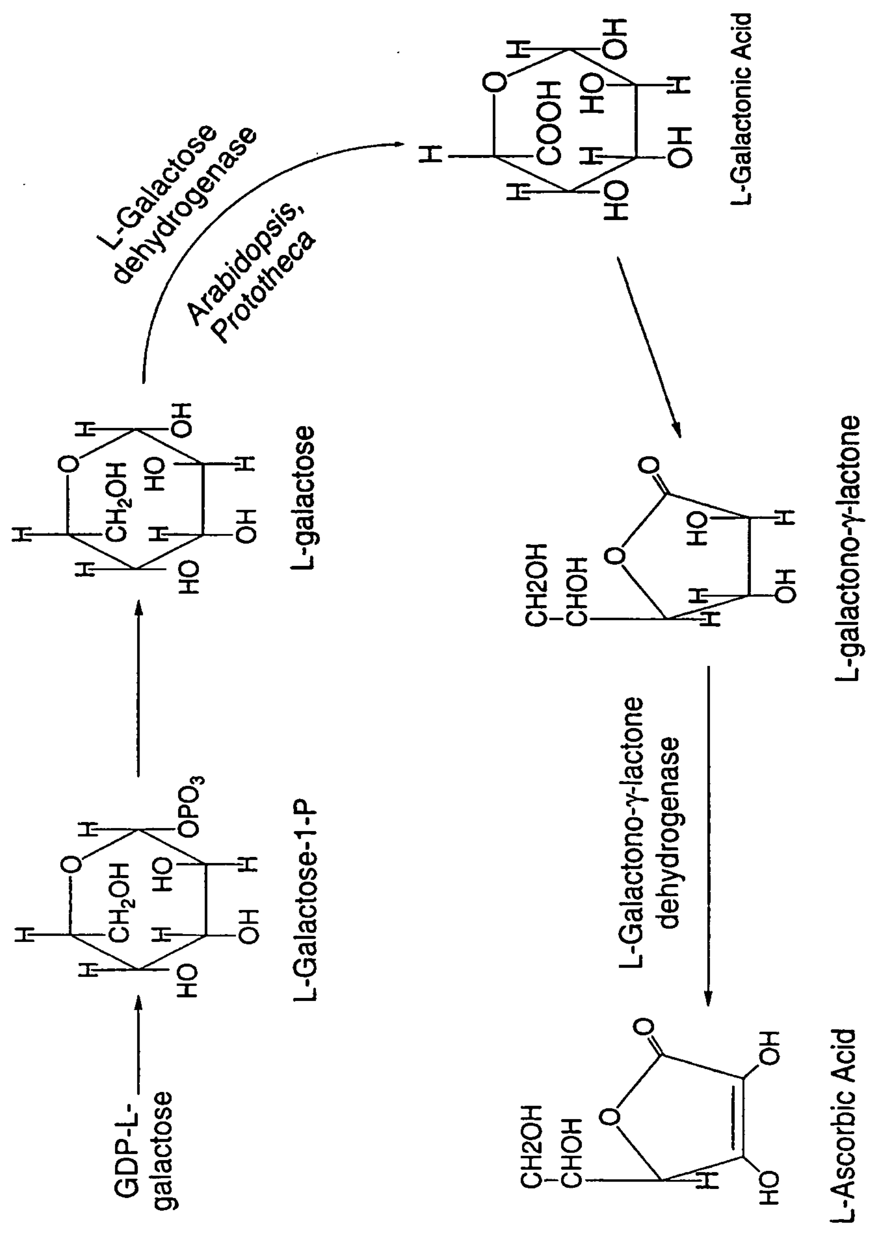

FIG. 1C

Selected Carbon Flow from Glucose in *Prototheca*

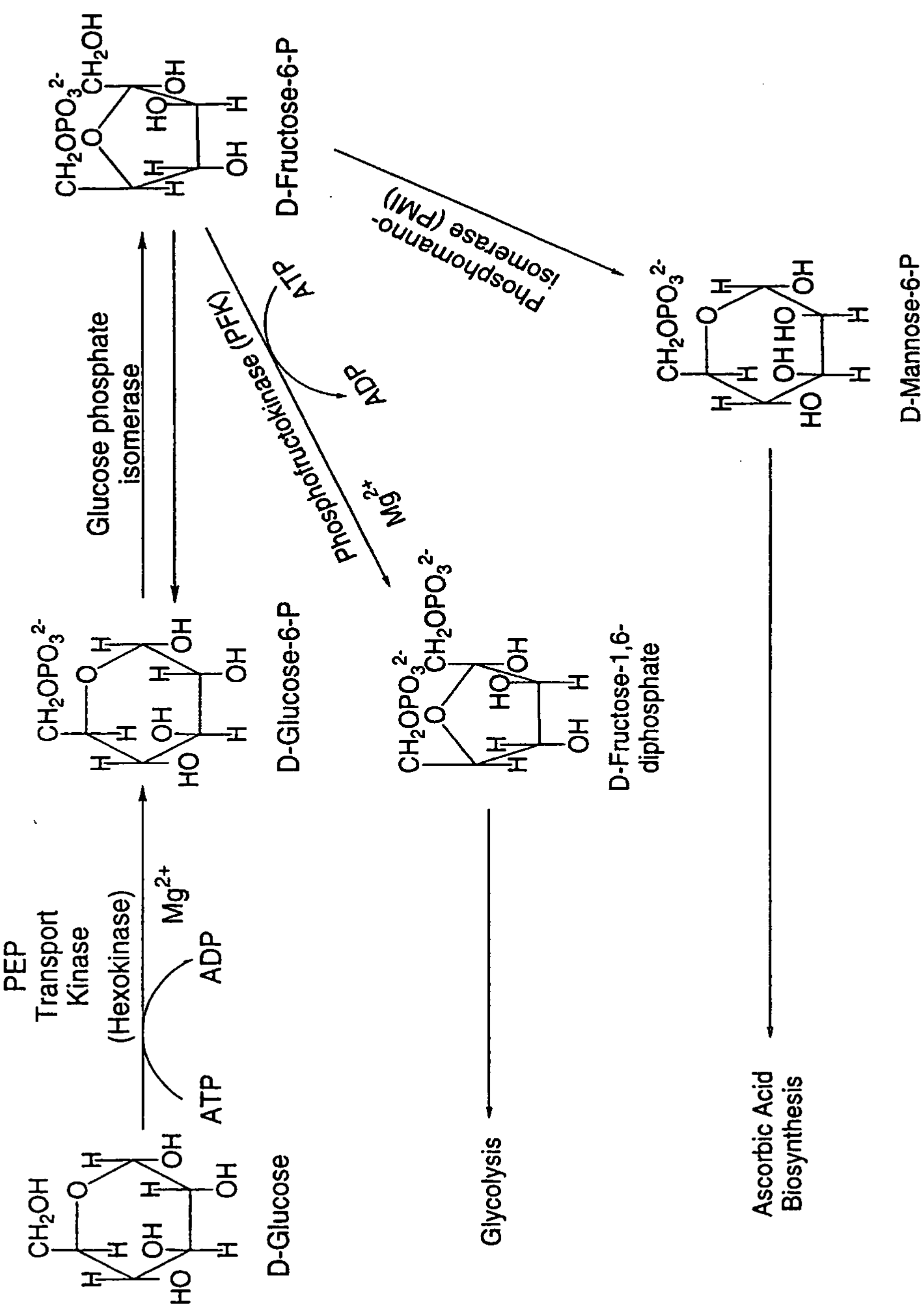

FIG. 2A

Selected Carbon Flow from Glucose in *Prototheca*, con't

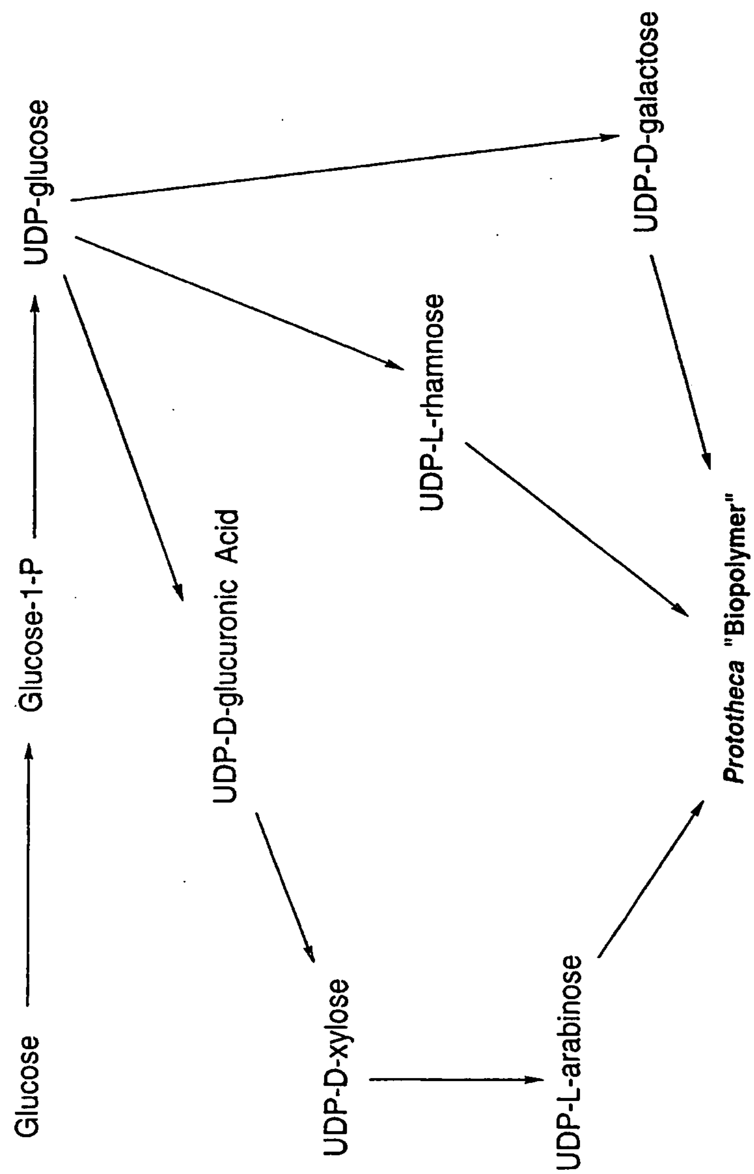

FIG. 2B

Genealogy of Selected Isolates

Specific Formations in Magnesium-Limited Secondary Shake Flasks after 96 Hours Incubation

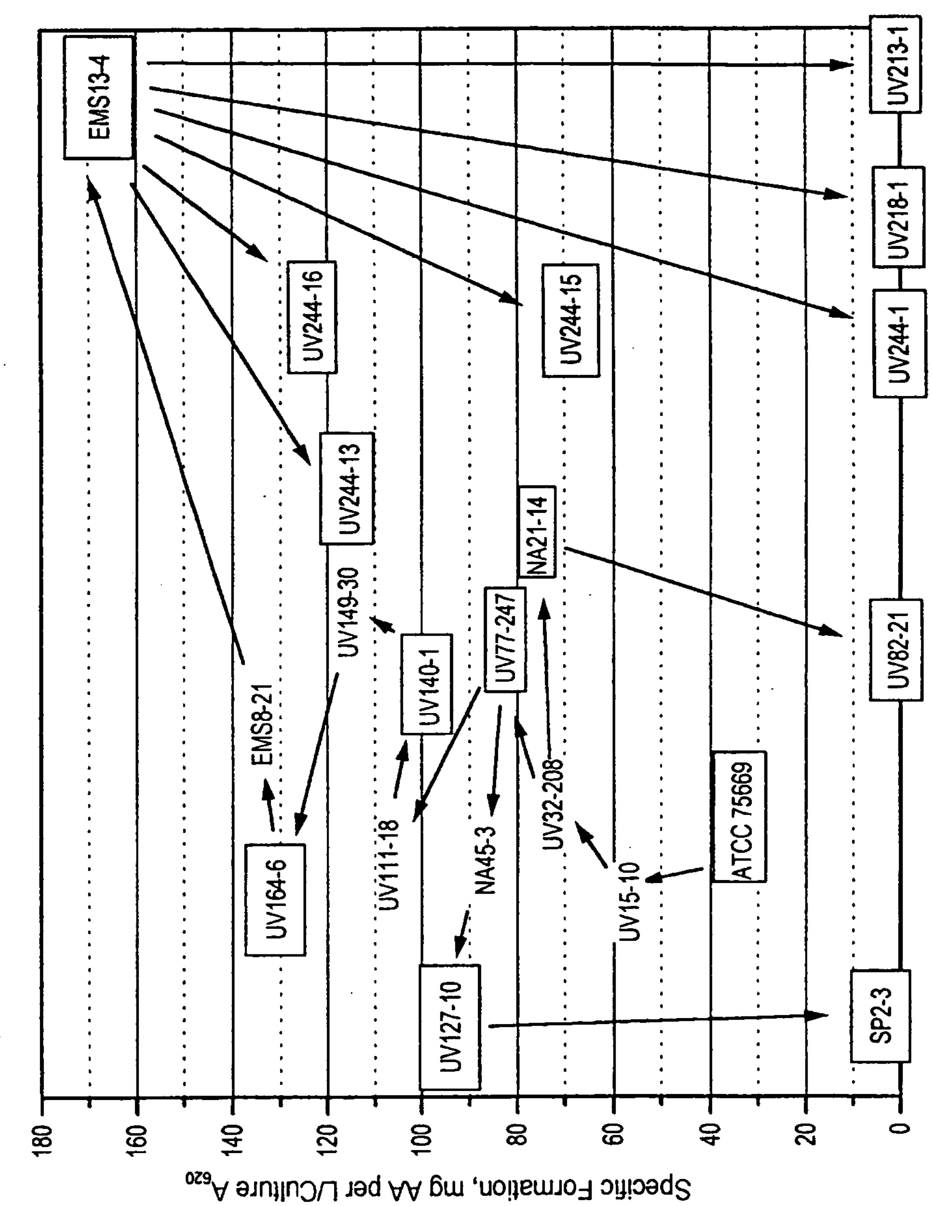

FIG. 3

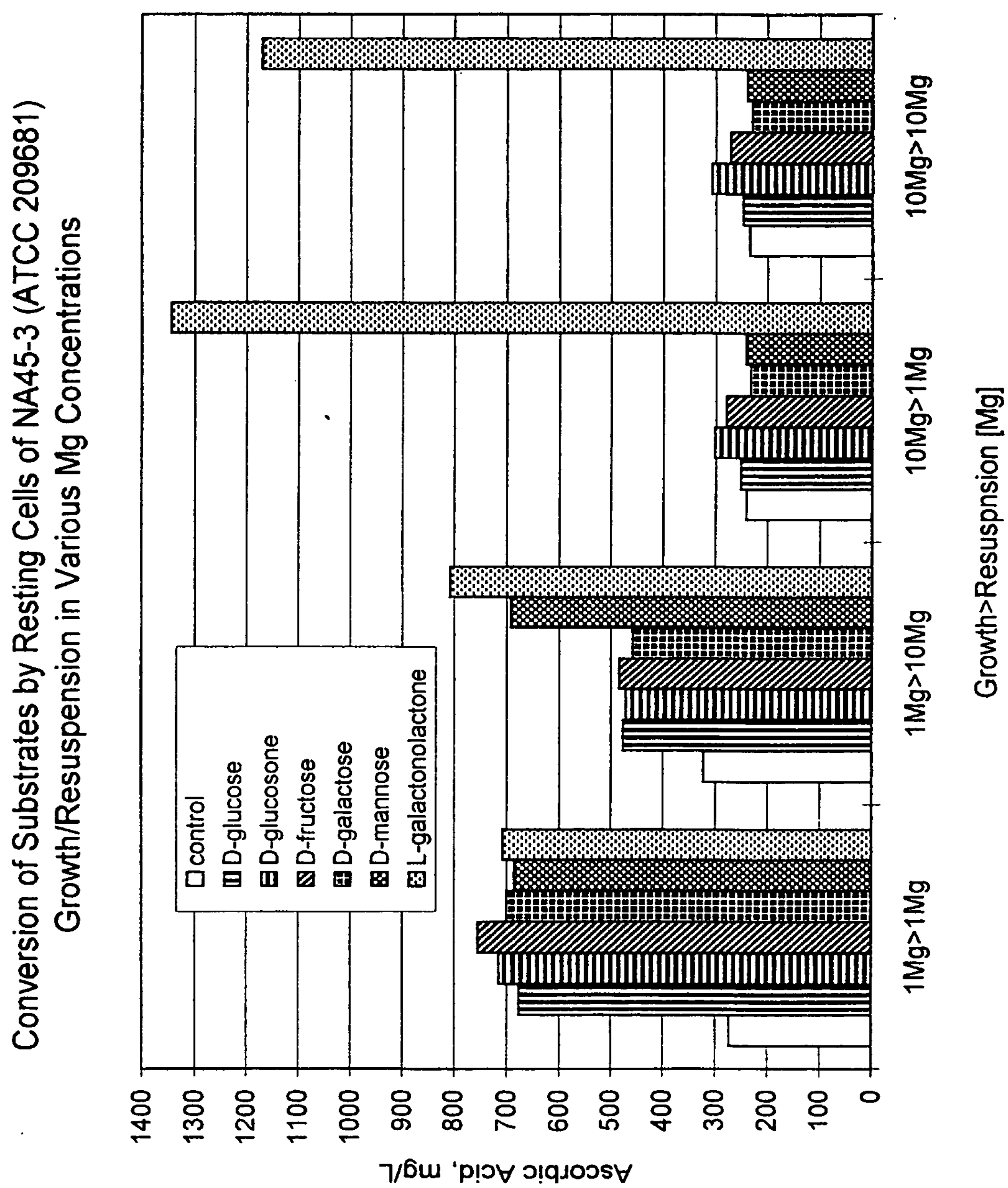
Conversion of Substrates by Resting Cells of NA45-3 (ATCC 209681)
Growth/Resuspension in Various Mg Concentrations
control
D-glucose
D-glucosone
D-fructose
D-galactose
D-mannose
L-galactonolactone
Ascorbic Acid, mg/L
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
1Mg>1Mg
1Mg>10Mg
10Mg>1Mg
10Mg>10Mg
Growth>Resuspnsion [Mg]

Fig. 4

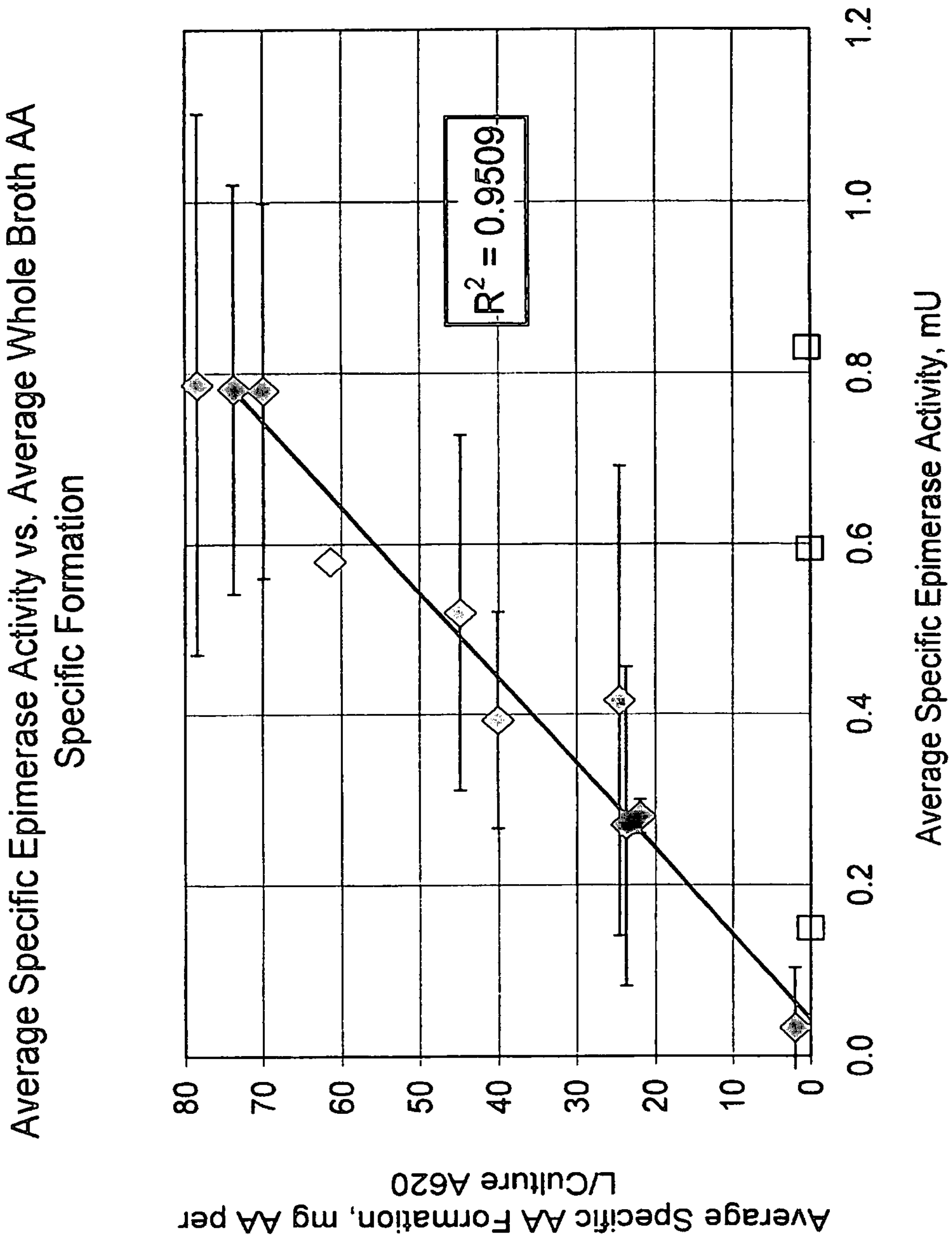
Average Specific Epimerase Activity vs. Average Whole Broth AA Specific Formation
$R^2 = 0.9509$
Average Specific AA Formation, mg AA per L/Culture A620
0
10
20
30
40
50
60
70
80
0.0
0.2
0.4
0.6
0.8
1.0
1.2
Average Specific Epimerase Activity, mU

Fig. 5

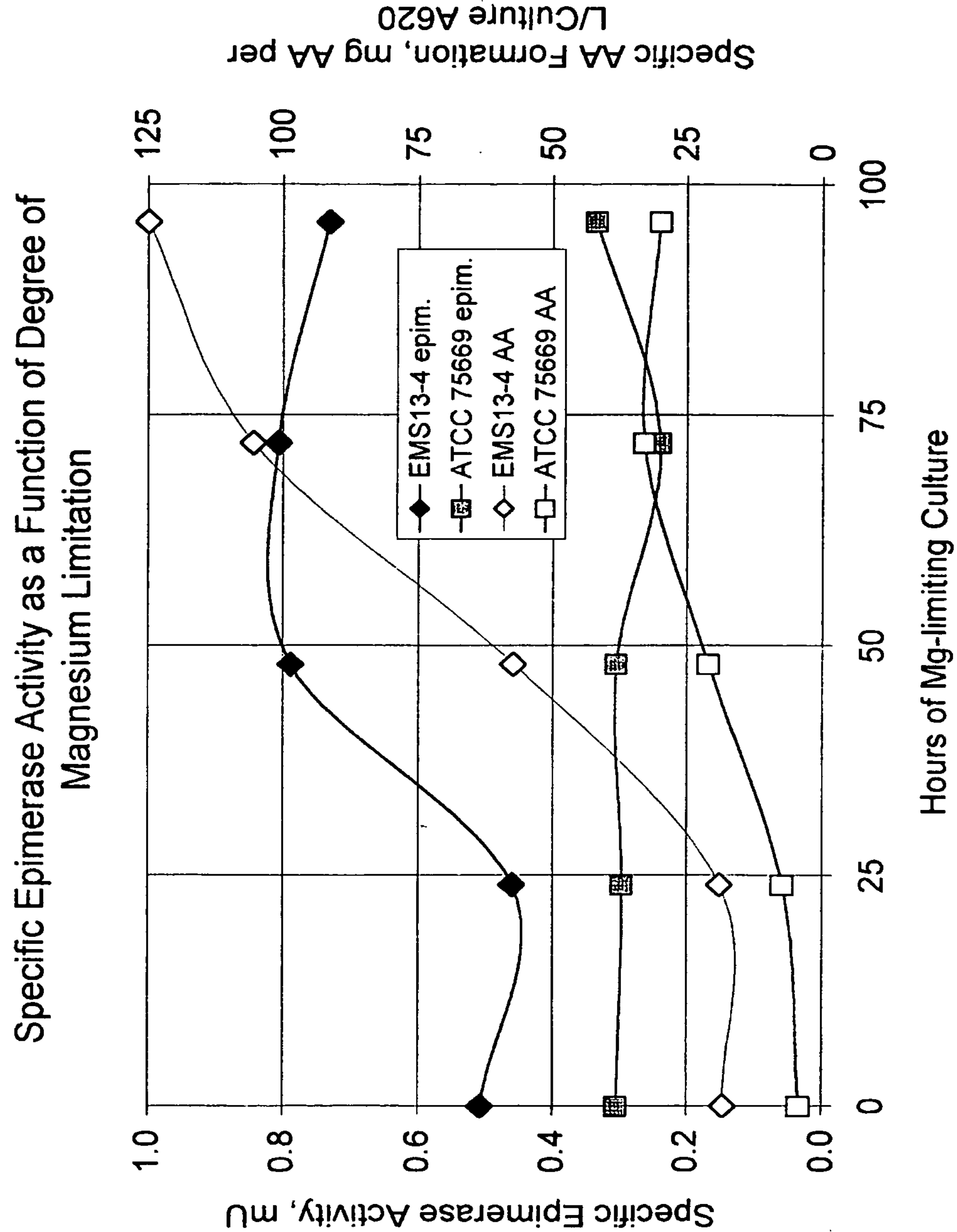
Specific Epimerase Activity as a Function of Degree of Magnesium Limitation
Specific Epimerase Activity, mU
1.0
0.8
0.6
0.4
0.2
0.0
Specific AA Formation, mg AA per L/Culture A620
125
100
75
50
25
0
0
25
50
75
100
Hours of Mg-limiting Culture
EMS13-4 epim.
ATCC 75669 epim.
EMS13-4 AA
ATCC 75669 AA

Fig. 6

Proposed Mechanism for the Conversion of GDP-D-mannose to GDP-L-galactose in *Chlorella pyrenoidosa* (Barber)

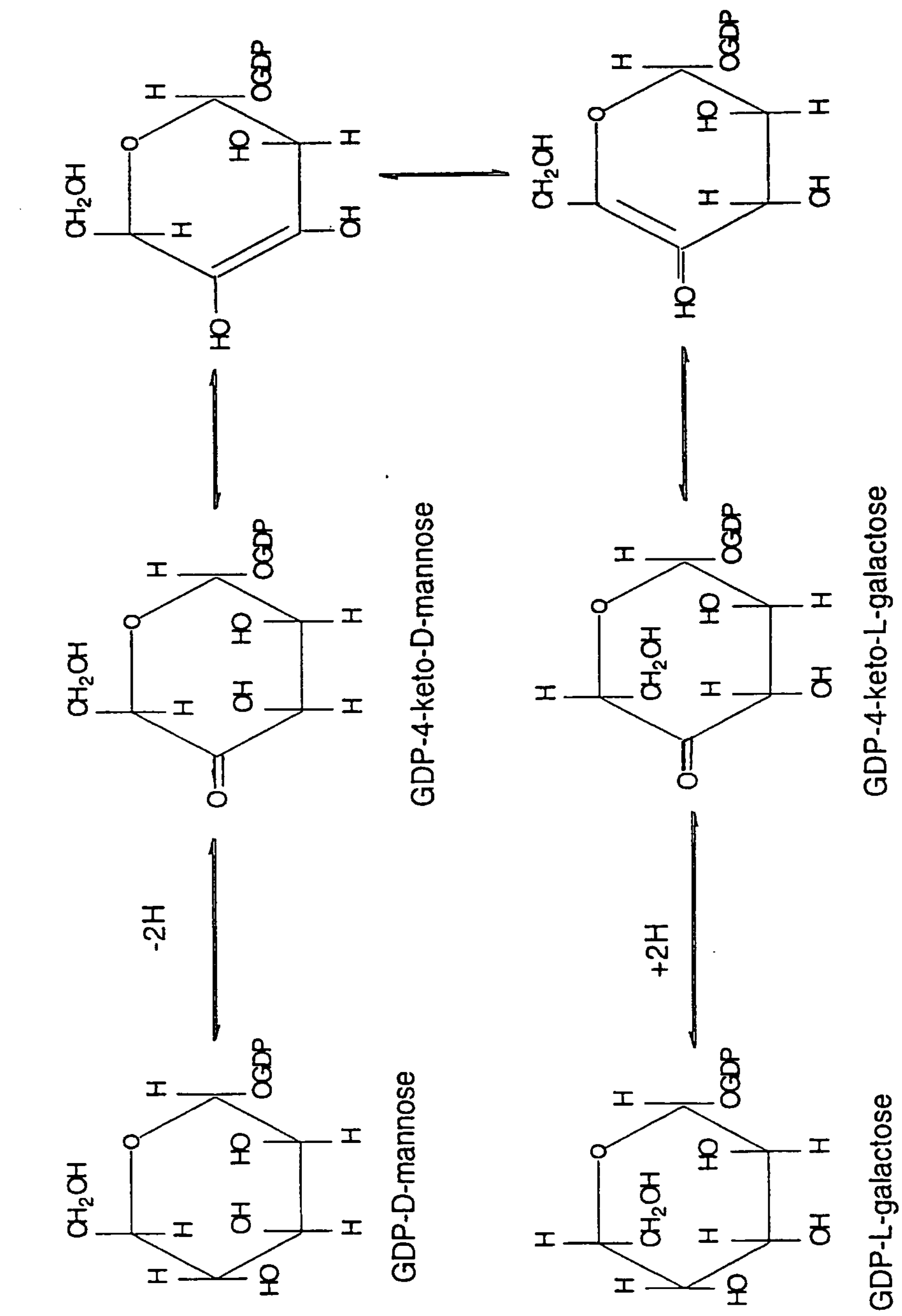

Fig. 7

Published Mechanism for the Conversion of GDP-D-mannose to GDP-4-keto-6-deoxy-D-mannose

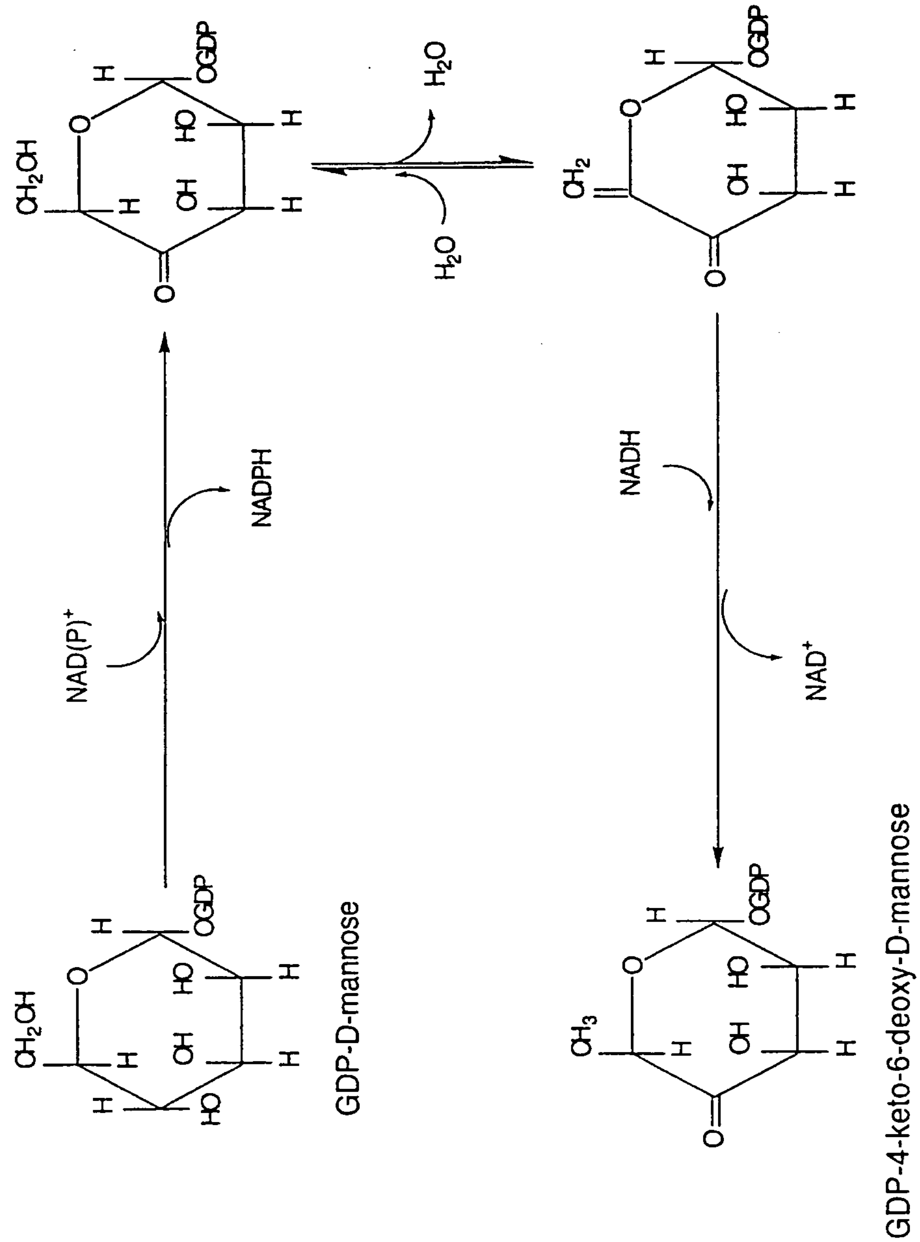

Fig. 8A

Published Mechanism for the Conversion of GDP-4-keto-6-deoxy-D-mannose to GDP-L-fucose

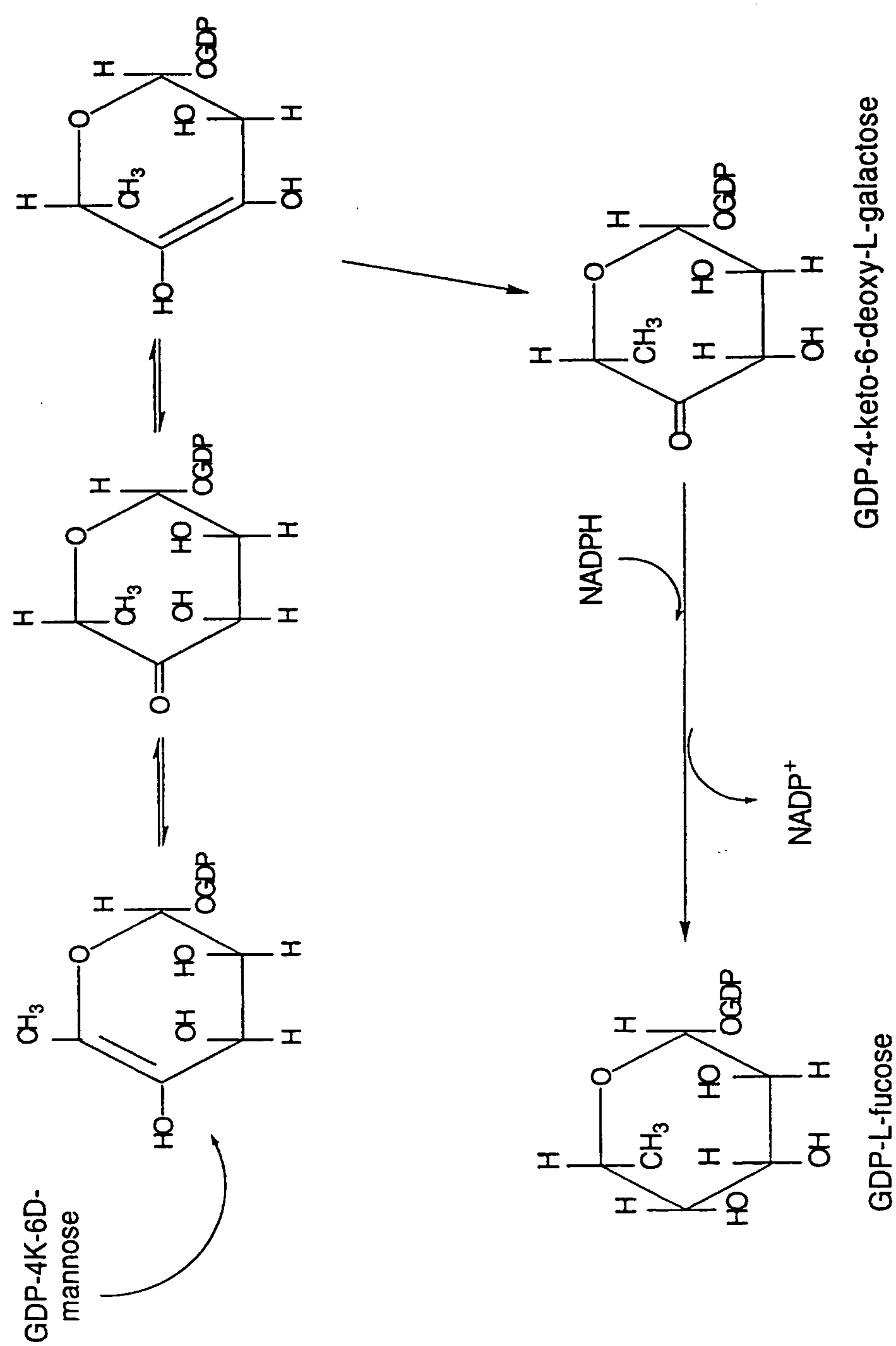

Fig. 8B

SEQUENCE LISTING

<110> Berry, Alan
Running, Jeffrey A.
Severson, David K.
Burlingame, Richard P.

<120> "VITAMIN C PRODUCTION IN MICROORGANISMS AND PLANTS"

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Val Met Pro Thr Asn Leu Tyr Gly Pro His Asp Asn Phe His Pro Ser
                165                 170                 175

aat tcg cat gtg atc cca gca ttg ctg cgt cgc ttc cac gag gcg acg    576
Asn Ser His Val Ile Pro Ala Leu Leu Arg Arg Phe His Glu Ala Thr
            180                 185                 190

gca cag aat gcg ccg gac gtg gtg gta tgg ggc agc ggt aca ccg atg    624
Ala Gln Asn Ala Pro Asp Val Val Val Trp Gly Ser Gly Thr Pro Met
        195                 200                 205

cgc gaa ttt ctg cac gtc gat gat atg gcg gcg gcg agc att cat gtc    672
Arg Glu Phe Leu His Val Asp Asp Met Ala Ala Ala Ser Ile His Val
    210                 215                 220

atg gag ctg gcg cat gaa gtc tgg ctg gag aac acc cag ccg atg ttg    720
Met Glu Leu Ala His Glu Val Trp Leu Glu Asn Thr Gln Pro Met Leu
225                 230                 235                 240

tcg cac att aac gtc ggc acg ggc gtt gac tgc act atc cgc gac gtg    768
Ser His Ile Asn Val Gly Thr Gly Val Asp Cys Thr Ile Arg Asp Val
                245                 250                 255

gcg caa acc atc gcc aaa gtg gtg ggt tac aaa ggc cgg gtg gtt ttt    816
Ala Gln Thr Ile Ala Lys Val Val Gly Tyr Lys Gly Arg Val Val Phe
            260                 265                 270

gat gcc agc aaa ccg gat ggc acg ccg cgc aaa ctg ctg gat gtg acg    864
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Asp Ala Ser Lys Pro Asp Gly Thr Pro Arg Lys Leu Leu Asp Val Thr
275 280 285

cgc ctg cat cag ctt ggc tgg tat cac gaa atc tca ctg gaa gcg ggg 912
Arg Leu His Gln Leu Gly Trp Tyr His Glu Ile Ser Leu Glu Ala Gly
290 295 300

ctt gcc agc act tac cag tgg ttc ctt gag aat caa gac cgc ttt cgg 960
Leu Ala Ser Thr Tyr Gln Trp Phe Leu Glu Asn Gln Asp Arg Phe Arg
305 310 315 320

ggg taa 966
Gly

<210> 4
<211> 321
<212> PRT
<213> Escherichia coli

<400> 4
Met Ser Lys Gln Arg Val Phe Ile Ala Gly His Arg Gly Met Val Gly
1 5 10 15

Ser Ala Ile Arg Arg Gln Leu Glu Gln Arg Gly Asp Val Glu Leu Val
20 25 30

Leu Arg Thr Arg Asp Glu Leu Asn Leu Leu Asp Ser Arg Ala Val His
35 40 45

Asp Phe Phe Ala Ser Glu Arg Ile Asp Gln Val Tyr Leu Ala Ala Ala
50 55 60

Lys Val Gly Gly Ile Val Ala Asn Asn Thr Tyr Pro Ala Asp Phe Ile
65 70 75 80

Tyr Gln Asn Met Met Ile Glu Ser Asn Ile Ile His Ala Ala His Gln
85 90 95

Asn Asp Val Asn Lys Leu Leu Phe Leu Gly Ser Ser Cys Ile Tyr Pro
100 105 110

Lys Leu Ala Lys Gln Pro Met Ala Glu Ser Glu Leu Leu Gln Gly Thr
115 120 125

Leu Glu Pro Thr Asn Glu Pro Tyr Ala Ile Ala Lys Ile Ala Gly Ile
130 135 140

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Lys Leu Cys Glu Ser Tyr Asn Arg Gln Tyr Gly Arg Asp Tyr Arg Ser
145                 150                 155                 160

Val Met Pro Thr Asn Leu Tyr Gly Pro His Asp Asn Phe His Pro Ser
                165                 170                 175

Asn Ser His Val Ile Pro Ala Leu Leu Arg Arg Phe His Glu Ala Thr
            180                 185                 190

Ala Gln Asn Ala Pro Asp Val Val Val Trp Gly Ser Gly Thr Pro Met
        195                 200                 205

Arg Glu Phe Leu His Val Asp Asp Met Ala Ala Ala Ser Ile His Val
    210                 215                 220

Met Glu Leu Ala His Glu Val Trp Leu Glu Asn Thr Gln Pro Met Leu
225                 230                 235                 240

Ser His Ile Asn Val Gly Thr Gly Val Asp Cys Thr Ile Arg Asp Val
                245                 250                 255

Ala Gln Thr Ile Ala Lys Val Val Gly Tyr Lys Gly Arg Val Val Phe
            260                 265                 270

Asp Ala Ser Lys Pro Asp Gly Thr Pro Arg Lys Leu Leu Asp Val Thr
        275                 280                 285

Arg Leu His Gln Leu Gly Trp Tyr His Glu Ile Ser Leu Glu Ala Gly
    290                 295                 300

Leu Ala Ser Thr Tyr Gln Trp Phe Leu Glu Asn Gln Asp Arg Phe Arg
305                 310                 315                 320

Gly


<210> 5
<211> 1340
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (75)..(1040)

<400> 5
ctagaattca gcggccgctg aattctagct agaattcagc ggccgctgaa ttctagaacc 60
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caggtgcaac tgac atg ggt gaa ccc cag gga tcc atg cgg att cta gtg    110
                Met Gly Glu Pro Gln Gly Ser Met Arg Ile Leu Val
                 1               5                  10

aca ggg ggc tct ggg ctg gta ggc aaa gcc atc cag aag gtg gta gca    158
Thr Gly Gly Ser Gly Leu Val Gly Lys Ala Ile Gln Lys Val Val Ala
        15                  20                  25

gat gga gct gga ctt cct gga gag gac tgg gtg ttt gtc tcc tct aaa    206
Asp Gly Ala Gly Leu Pro Gly Glu Asp Trp Val Phe Val Ser Ser Lys
    30                  35                  40

gac gcc gat ctc acg gat aca gca cag acc cgc gcc ctg ttt gag aag    254
Asp Ala Asp Leu Thr Asp Thr Ala Gln Thr Arg Ala Leu Phe Glu Lys
 45                  50                  55                  60

gtc caa ccc aca cac gtc atc cat ctt gct gca atg gtg ggg ggc ctg    302
Val Gln Pro Thr His Val Ile His Leu Ala Ala Met Val Gly Gly Leu
                 65                  70                  75

ttc cgg aat atc aaa tac aat ttg gac ttc tgg agg aaa aac gtg cac    350
Phe Arg Asn Ile Lys Tyr Asn Leu Asp Phe Trp Arg Lys Asn Val His
             80                  85                  90

atg aac gac aac gtc ctg cac tcg gcc ttt gag gtg ggg gcc cgc aag    398
Met Asn Asp Asn Val Leu His Ser Ala Phe Glu Val Gly Ala Arg Lys
        95                 100                 105

gtg gtg tcc tgc ctg tcc acc tgt atc ttc cct gac aag acg acc tac    446
Val Val Ser Cys Leu Ser Thr Cys Ile Phe Pro Asp Lys Thr Thr Tyr
    110                 115                 120

ccg ata gat gag acc atg atc cac aat ggg cct ccc cac aac agc aat    494
Pro Ile Asp Glu Thr Met Ile His Asn Gly Pro Pro His Asn Ser Asn
125                 130                 135                 140

ttt ggg tac tcg tat gcc aag agg atg atc gac gtg cag aac agg gcc    542
Phe Gly Tyr Ser Tyr Ala Lys Arg Met Ile Asp Val Gln Asn Arg Ala
                145                 150                 155

tac ttc cag cag tac ggc tgc acc ttc acc gct gtc atc ccc acc aac    590
Tyr Phe Gln Gln Tyr Gly Cys Thr Phe Thr Ala Val Ile Pro Thr Asn
            160                 165                 170

gtt ttc ggg ccc cac gac aac ttc aac atc gag gat ggc cac gtg ctg    638
Val Phe Gly Pro His Asp Asn Phe Asn Ile Glu Asp Gly His Val Leu
        175                 180                 185
```

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cct ggc ctc atc cac aag gtg cac ctg gcc aag agc agc ggc tcg gcc    686
Pro Gly Leu Ile His Lys Val His Leu Ala Lys Ser Ser Gly Ser Ala
    190                 195                 200

ctg acg gtg tgg ggt aca ggg aat ccg cgg agg cag ttc ata tac tcg    734
Leu Thr Val Trp Gly Thr Gly Asn Pro Arg Arg Gln Phe Ile Tyr Ser
205                 210                 215                 220

ctg gac ctg gcc cag ctc ttt atc tgg gtc ctg cgg gag tac aat gaa    782
Leu Asp Leu Ala Gln Leu Phe Ile Trp Val Leu Arg Glu Tyr Asn Glu
                225                 230                 235

gtg gag ccc atc atc ctc tcc gtg ggc gag gaa gat gag gtc tcc atc    830
Val Glu Pro Ile Ile Leu Ser Val Gly Glu Glu Asp Glu Val Ser Ile
            240                 245                 250

aag gag gca gcc gag gcg gtg gtg gag gcc atg gac ttc cat ggg gaa    878
Lys Glu Ala Ala Glu Ala Val Val Glu Ala Met Asp Phe His Gly Glu
        255                 260                 265

gtc acc ttt gat aca acc aag tcg gat ggg cag ttt aag aag aca gcc    926
Val Thr Phe Asp Thr Thr Lys Ser Asp Gly Gln Phe Lys Lys Thr Ala
    270                 275                 280

agt aac agc aag ctg agg acc tac ctg ccc gac ttc cgg ttc aca ccc    974
Ser Asn Ser Lys Leu Arg Thr Tyr Leu Pro Asp Phe Arg Phe Thr Pro
285                 290                 295                 300

ttc aag cag gcg gtg aag gag acc tgt gct tgg ttc act gac aac tac    1022
Phe Lys Gln Ala Val Lys Glu Thr Cys Ala Trp Phe Thr Asp Asn Tyr
                305                 310                 315

gag cag gcc cgg aag tga agctggaaga caggatcagg tgccagcgga           1070
Glu Gln Ala Arg Lys
            320

ccatcggctg gcagagccca gcggccacca cccgtcaacc ctgccaggag ctgagggcac 1130

cacccagcaa cctgggcctg cattccatcc gctctgcagc cccaagcatc tttccagtgg 1190

ggcccccatt cacgttggtc ctcagggaaa ccagggtccg gggcaggccc ggcgctttgc 1250

tccccacacc agccccctgc gcgtgtccac tctgatcctg catcccactc cctgggagcc 1310

aataaagtgc attttcacag aaaaaaaaaa                                   1340
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<210> 6

<211> 321
<212> PRT
<213> Homo sapiens

<400> 6
Met Gly Glu Pro Gln Gly Ser Met Arg Ile Leu Val Thr Gly Gly Ser
1 5 10 15

Gly Leu Val Gly Lys Ala Ile Gln Lys Val Val Ala Asp Gly Ala Gly
20 25 30

Leu Pro Gly Glu Asp Trp Val Phe Val Ser Ser Lys Asp Ala Asp Leu
35 40 45

Thr Asp Thr Ala Gln Thr Arg Ala Leu Phe Glu Lys Val Gln Pro Thr
50 55 60

His Val Ile His Leu Ala Ala Met Val Gly Gly Leu Phe Arg Asn Ile
65 70 75 80

Lys Tyr Asn Leu Asp Phe Trp Arg Lys Asn Val His Met Asn Asp Asn
85 90 95

Val Leu His Ser Ala Phe Glu Val Gly Ala Arg Lys Val Val Ser Cys
100 105 110

Leu Ser Thr Cys Ile Phe Pro Asp Lys Thr Thr Tyr Pro Ile Asp Glu
115 120 125

Thr Met Ile His Asn Gly Pro Pro His Asn Ser Asn Phe Gly Tyr Ser
130 135 140

Tyr Ala Lys Arg Met Ile Asp Val Gln Asn Arg Ala Tyr Phe Gln Gln
145 150 155 160

Tyr Gly Cys Thr Phe Thr Ala Val Ile Pro Thr Asn Val Phe Gly Pro
165 170 175

His Asp Asn Phe Asn Ile Glu Asp Gly His Val Leu Pro Gly Leu Ile
180 185 190

His Lys Val His Leu Ala Lys Ser Ser Gly Ser Ala Leu Thr Val Trp
195 200 205

Gly Thr Gly Asn Pro Arg Arg Gln Phe Ile Tyr Ser Leu Asp Leu Ala
210 215 220

Gln Leu Phe Ile Trp Val Leu Arg Glu Tyr Asn Glu Val Glu Pro Ile

225 230 235 240

Ile Leu Ser Val Gly Glu Glu Asp Glu Val Ser Ile Lys Glu Ala Ala
245 250 255

Glu Ala Val Val Glu Ala Met Asp Phe His Gly Glu Val Thr Phe Asp
260 265 270

Thr Thr Lys Ser Asp Gly Gln Phe Lys Lys Thr Ala Ser Asn Ser Lys
275 280 285

Leu Arg Thr Tyr Leu Pro Asp Phe Arg Phe Thr Pro Phe Lys Gln Ala
290 295 300

Val Lys Glu Thr Cys Ala Trp Phe Thr Asp Asn Tyr Glu Gln Ala Arg
305 310 315 320

Lys

<210> 7
<211> 1017
<212> DNA
<213> Escherichia coli

<220>
<221> CDS
<222> (1)..(1017)

<400> 7
atg aga gtt ctg gtt acc ggt ggt agc ggt tac att gga agt cat acc 48
Met Arg Val Leu Val Thr Gly Gly Ser Gly Tyr Ile Gly Ser His Thr
1 5 10 15

tgt gtg caa tta ctg caa aac ggt cat gat gtc atc att ctt gat aac 96
Cys Val Gln Leu Leu Gln Asn Gly His Asp Val Ile Ile Leu Asp Asn
20 25 30

ctc tgt aac agt aag cgc agc gta ctg cct gtt atc gag cgt tta ggc 144
Leu Cys Asn Ser Lys Arg Ser Val Leu Pro Val Ile Glu Arg Leu Gly
35 40 45

ggc aaa cat cca acg ttt gtt gaa ggc gat att cgt aac gaa gcg ttg 192
Gly Lys His Pro Thr Phe Val Glu Gly Asp Ile Arg Asn Glu Ala Leu
50 55 60

atg acc gag atc ctg cac gat cac gct atc gac acc gtg atc cac ttc 240
Met Thr Glu Ile Leu His Asp His Ala Ile Asp Thr Val Ile His Phe

65 70 75 80

gcc ggg ctg aaa gcc gtg ggc gaa tcg gta caa aaa ccg ctg gaa tat 288
Ala Gly Leu Lys Ala Val Gly Glu Ser Val Gln Lys Pro Leu Glu Tyr
85 90 95

tac gac aac aat gtc aac ggc act ctg cgc ctg att agc gcc atg cgc 336
Tyr Asp Asn Asn Val Asn Gly Thr Leu Arg Leu Ile Ser Ala Met Arg
100 105 110

gcc gct aac gtc aaa aac ttt att ttt agc tcc tcc gcc acc gtt tat 384
Ala Ala Asn Val Lys Asn Phe Ile Phe Ser Ser Ser Ala Thr Val Tyr
115 120 125

ggc gat cag ccc aaa att cca tac gtt gaa agc ttc ccg acc ggc aca 432
Gly Asp Gln Pro Lys Ile Pro Tyr Val Glu Ser Phe Pro Thr Gly Thr
130 135 140

ccg caa agc cct tac ggc aaa agc aag ctg atg gtg gaa cag atc ctc 480
Pro Gln Ser Pro Tyr Gly Lys Ser Lys Leu Met Val Glu Gln Ile Leu
145 150 155 160

acc gat ctg caa aaa gcc cag ccg gac tgg agc att gcc ctg ctg cgc 528
Thr Asp Leu Gln Lys Ala Gln Pro Asp Trp Ser Ile Ala Leu Leu Arg
165 170 175

tac ttc aac ccg gtt ggc gcg cat ccg tcg ggc gat atg ggc gaa gat 576
Tyr Phe Asn Pro Val Gly Ala His Pro Ser Gly Asp Met Gly Glu Asp
180 185 190

ccg caa ggc att ccg aat aac ctg atg cca tac atc gcc cag gtt gct 624
Pro Gln Gly Ile Pro Asn Asn Leu Met Pro Tyr Ile Ala Gln Val Ala
195 200 205

gta ggc cgt cgc gac tcg ctg gcg att ttt ggt aac gat tat ccg acc 672
Val Gly Arg Arg Asp Ser Leu Ala Ile Phe Gly Asn Asp Tyr Pro Thr
210 215 220

gaa gat ggt act ggc gta cgc gat tac atc cac gta atg gat ctg gcg 720
Glu Asp Gly Thr Gly Val Arg Asp Tyr Ile His Val Met Asp Leu Ala
225 230 235 240

gac ggt cac gtc gtg gcg atg gaa aaa ctg gcg aac aag cca ggc gta 768
Asp Gly His Val Val Ala Met Glu Lys Leu Ala Asn Lys Pro Gly Val
245 250 255

cac atc tac aac ctc ggc gct ggc gta ggc aac agc gtg ctg gac gtg 816
His Ile Tyr Asn Leu Gly Ala Gly Val Gly Asn Ser Val Leu Asp Val

260 265 270

gtt aat gcc ttc agc aaa gcc tgc ggc aaa ccg gtt aat tat cat ttt 864
Val Asn Ala Phe Ser Lys Ala Cys Gly Lys Pro Val Asn Tyr His Phe
275 280 285

gca ccg cgt cgc gag ggc gac ctt ccg gcc tac tgg gcg gac gcc agc 912
Ala Pro Arg Arg Glu Gly Asp Leu Pro Ala Tyr Trp Ala Asp Ala Ser
290 295 300

aaa gcc gac cgt gaa ctg aac tgg cgc gta acg cgc aca ctc gat gaa 960
Lys Ala Asp Arg Glu Leu Asn Trp Arg Val Thr Arg Thr Leu Asp Glu
305 310 315 320

atg gcg cag gac acc tgg cac tgg cag tca cgc cat cca cag gga tat 1008
Met Ala Gln Asp Thr Trp His Trp Gln Ser Arg His Pro Gln Gly Tyr
325 330 335

ccc gat taa 1017
Pro Asp

<210> 8
<211> 338
<212> PRT
<213> Escherichia coli

<400> 8
Met Arg Val Leu Val Thr Gly Gly Ser Gly Tyr Ile Gly Ser His Thr
1 5 10 15

Cys Val Gln Leu Leu Gln Asn Gly His Asp Val Ile Ile Leu Asp Asn
20 25 30

Leu Cys Asn Ser Lys Arg Ser Val Leu Pro Val Ile Glu Arg Leu Gly
35 40 45

Gly Lys His Pro Thr Phe Val Glu Gly Asp Ile Arg Asn Glu Ala Leu
50 55 60

Met Thr Glu Ile Leu His Asp His Ala Ile Asp Thr Val Ile His Phe
65 70 75 80

Ala Gly Leu Lys Ala Val Gly Glu Ser Val Gln Lys Pro Leu Glu Tyr
85 90 95

Tyr Asp Asn Asn Val Asn Gly Thr Leu Arg Leu Ile Ser Ala Met Arg
100 105 110

Ala Ala Asn Val Lys Asn Phe Ile Phe Ser Ser Ser Ala Thr Val Tyr
115 120 125

Gly Asp Gln Pro Lys Ile Pro Tyr Val Glu Ser Phe Pro Thr Gly Thr
130 135 140

Pro Gln Ser Pro Tyr Gly Lys Ser Lys Leu Met Val Glu Gln Ile Leu
145 150 155 160

Thr Asp Leu Gln Lys Ala Gln Pro Asp Trp Ser Ile Ala Leu Leu Arg
165 170 175

Tyr Phe Asn Pro Val Gly Ala His Pro Ser Gly Asp Met Gly Glu Asp
180 185 190

Pro Gln Gly Ile Pro Asn Asn Leu Met Pro Tyr Ile Ala Gln Val Ala
195 200 205

Val Gly Arg Arg Asp Ser Leu Ala Ile Phe Gly Asn Asp Tyr Pro Thr
210 215 220

Glu Asp Gly Thr Gly Val Arg Asp Tyr Ile His Val Met Asp Leu Ala
225 230 235 240

Asp Gly His Val Val Ala Met Glu Lys Leu Ala Asn Lys Pro Gly Val
245 250 255

His Ile Tyr Asn Leu Gly Ala Gly Val Gly Asn Ser Val Leu Asp Val
260 265 270

Val Asn Ala Phe Ser Lys Ala Cys Gly Lys Pro Val Asn Tyr His Phe
275 280 285

Ala Pro Arg Arg Glu Gly Asp Leu Pro Ala Tyr Trp Ala Asp Ala Ser
290 295 300

Lys Ala Asp Arg Glu Leu Asn Trp Arg Val Thr Arg Thr Leu Asp Glu
305 310 315 320

Met Ala Gln Asp Thr Trp His Trp Gln Ser Arg His Pro Gln Gly Tyr
325 330 335

Pro Asp

<210> 9
<211> 1047

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<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(1047)

<400> 9
atg gca gag aag gtg ctg gta aca ggt ggg gct ggc tac att ggc agc    48
Met Ala Glu Lys Val Leu Val Thr Gly Gly Ala Gly Tyr Ile Gly Ser
  1               5                  10                  15

cac acg gtg ctg gag ctg ctg gag gct ggc tac ttg cct gtg gtc atc    96
His Thr Val Leu Glu Leu Leu Glu Ala Gly Tyr Leu Pro Val Val Ile
             20                  25                  30

gat aac ttc cat aat gcc ttc cgt gga ggg ggc tcc ctg cct gag agc   144
Asp Asn Phe His Asn Ala Phe Arg Gly Gly Gly Ser Leu Pro Glu Ser
         35                  40                  45

ctg cgg cgg gtc cag gag ctg aca ggc cgc tct gtg gag ttt gag gag   192
Leu Arg Arg Val Gln Glu Leu Thr Gly Arg Ser Val Glu Phe Glu Glu
     50                  55                  60

atg gac att ttg gac cag gga gcc cta cag cgt ctc ttc aaa aag tac   240
Met Asp Ile Leu Asp Gln Gly Ala Leu Gln Arg Leu Phe Lys Lys Tyr
 65                  70                  75                  80

agc ttt atg gcg gtc atc cac ttt gcg ggg ctc aag gcc gtg ggc gag   288
Ser Phe Met Ala Val Ile His Phe Ala Gly Leu Lys Ala Val Gly Glu
                 85                  90                  95

tcg gtg cag aag cct ctg gat tat tac aga gtt aac ctg acc ggg acc   336
Ser Val Gln Lys Pro Leu Asp Tyr Tyr Arg Val Asn Leu Thr Gly Thr
            100                 105                 110

atc cag ctt ctg gag atc atg aag gcc cac ggg gtg aag aac ctg gtg   384
Ile Gln Leu Leu Glu Ile Met Lys Ala His Gly Val Lys Asn Leu Val
        115                 120                 125

ttc agc agc tca gcc act gtg tac ggg aac ccc cag tac ctg ccc ctt   432
Phe Ser Ser Ser Ala Thr Val Tyr Gly Asn Pro Gln Tyr Leu Pro Leu
    130                 135                 140

gat gag gcc cac ccc acg ggt ggt tgt acc aac cct tac ggc aag tcc   480
Asp Glu Ala His Pro Thr Gly Gly Cys Thr Asn Pro Tyr Gly Lys Ser
145                 150                 155                 160
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aag ttc ttc atc gag gaa atg atc cgg gac ctg tgc cag gca gac aag    528
Lys Phe Phe Ile Glu Glu Met Ile Arg Asp Leu Cys Gln Ala Asp Lys
                165                 170                 175

act tgg aac gta gtg ctg ctg cgc tat ttc aac ccc aca ggt gcc cat    576
Thr Trp Asn Val Val Leu Leu Arg Tyr Phe Asn Pro Thr Gly Ala His
            180                 185                 190

gcc tct ggc tgc att ggt gag gat ccc cag ggc ata ccc aac aac ctc    624
Ala Ser Gly Cys Ile Gly Glu Asp Pro Gln Gly Ile Pro Asn Asn Leu
        195                 200                 205

atg cct tat gtc tcc cag gtg gcg atc ggg cga cgg gag gcc ctg aat    672
Met Pro Tyr Val Ser Gln Val Ala Ile Gly Arg Arg Glu Ala Leu Asn
    210                 215                 220

gtc ttt ggc aat gac tat gac aca gag gat ggc aca ggt gtc cgg gat    720
Val Phe Gly Asn Asp Tyr Asp Thr Glu Asp Gly Thr Gly Val Arg Asp
225                 230                 235                 240

tac atc cat gtc gtg gat ctg gcc aag ggc cac att gca gcc tta agg    768
Tyr Ile His Val Val Asp Leu Ala Lys Gly His Ile Ala Ala Leu Arg
                245                 250                 255

aag ctg aaa gaa cag tgt ggc tgc cgg atc tac aac ctg ggc acg ggc    816
Lys Leu Lys Glu Gln Cys Gly Cys Arg Ile Tyr Asn Leu Gly Thr Gly
            260                 265                 270

aca ggc tat tca gtg ctg cag atg gtc cag gct atg gag aag gcc tct    864
Thr Gly Tyr Ser Val Leu Gln Met Val Gln Ala Met Glu Lys Ala Ser
        275                 280                 285

ggg aag aag atc ccg tac aag gtg gtg gca cgg cgg gaa ggt gat gtg    912
Gly Lys Lys Ile Pro Tyr Lys Val Val Ala Arg Arg Glu Gly Asp Val
    290                 295                 300

gca gcc tgt tac gcc aac ccc agc ctg gcc caa gag gag ctg ggg tgg    960
Ala Ala Cys Tyr Ala Asn Pro Ser Leu Ala Gln Glu Glu Leu Gly Trp
305                 310                 315                 320

aca gca gcc tta ggg ctg gac agg atg tgt gag gat ctc tgg cgc tgg    1008
Thr Ala Ala Leu Gly Leu Asp Arg Met Cys Glu Asp Leu Trp Arg Trp
                325                 330                 335

cag aag cag aat cct tca ggc ttt ggc acg caa gcc tga                1047
Gln Lys Gln Asn Pro Ser Gly Phe Gly Thr Gln Ala
            340                 345
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<210> 10
<211> 348
<212> PRT
<213> Homo sapiens

<400> 10
Met Ala Glu Lys Val Leu Val Thr Gly Gly Ala Gly Tyr Ile Gly Ser
 1               5                  10                  15

His Thr Val Leu Glu Leu Leu Glu Ala Gly Tyr Leu Pro Val Val Ile
            20                  25                  30

Asp Asn Phe His Asn Ala Phe Arg Gly Gly Gly Ser Leu Pro Glu Ser
        35                  40                  45

Leu Arg Arg Val Gln Glu Leu Thr Gly Arg Ser Val Glu Phe Glu Glu
    50                  55                  60

Met Asp Ile Leu Asp Gln Gly Ala Leu Gln Arg Leu Phe Lys Lys Tyr
65                  70                  75                  80

Ser Phe Met Ala Val Ile His Phe Ala Gly Leu Lys Ala Val Gly Glu
                85                  90                  95

Ser Val Gln Lys Pro Leu Asp Tyr Tyr Arg Val Asn Leu Thr Gly Thr
            100                 105                 110

Ile Gln Leu Leu Glu Ile Met Lys Ala His Gly Val Lys Asn Leu Val
        115                 120                 125

Phe Ser Ser Ser Ala Thr Val Tyr Gly Asn Pro Gln Tyr Leu Pro Leu
    130                 135                 140

Asp Glu Ala His Pro Thr Gly Gly Cys Thr Asn Pro Tyr Gly Lys Ser
145                 150                 155                 160

Lys Phe Phe Ile Glu Glu Met Ile Arg Asp Leu Cys Gln Ala Asp Lys
                165                 170                 175

Thr Trp Asn Val Val Leu Leu Arg Tyr Phe Asn Pro Thr Gly Ala His
            180                 185                 190

Ala Ser Gly Cys Ile Gly Glu Asp Pro Gln Gly Ile Pro Asn Asn Leu
        195                 200                 205

Met Pro Tyr Val Ser Gln Val Ala Ile Gly Arg Arg Glu Ala Leu Asn
    210                 215                 220
```

Val Phe Gly Asn Asp Tyr Asp Thr Glu Asp Gly Thr Gly Val Arg Asp
225 230 235 240

Tyr Ile His Val Val Asp Leu Ala Lys Gly His Ile Ala Ala Leu Arg
245 250 255

Lys Leu Lys Glu Gln Cys Gly Cys Arg Ile Tyr Asn Leu Gly Thr Gly
260 265 270

Thr Gly Tyr Ser Val Leu Gln Met Val Gln Ala Met Glu Lys Ala Ser
275 280 285

Gly Lys Lys Ile Pro Tyr Lys Val Val Ala Arg Arg Glu Gly Asp Val
290 295 300

Ala Ala Cys Tyr Ala Asn Pro Ser Leu Ala Gln Glu Glu Leu Gly Trp
305 310 315 320

Thr Ala Ala Leu Gly Leu Asp Arg Met Cys Glu Asp Leu Trp Arg Trp
325 330 335

Gln Lys Gln Asn Pro Ser Gly Phe Gly Thr Gln Ala
340 345

<210> 11
<211> 317
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:CONSENSUS

<400> 11
Xaa Xaa Arg Xaa Xaa Xaa Xaa Gly Xaa Xaa Gly Xaa Xaa Gly Xaa Xaa
1 5 10 15

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
20 25 30

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
35 40 45

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Val Xaa Xaa Xaa Ala Xaa Xaa Xaa
50 55 60

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa

65 70 75 80

Asn Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
85 90 95

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Ser Xaa Xaa Xaa Xaa Xaa Xaa Xaa
100 105 110

Xaa Xaa Xaa Pro Xaa Xaa Glu Xaa Xaa Xaa Xaa Xaa Gly Xaa Xaa Xaa
115 120 125

Xaa Xaa Xaa Xaa Xaa Tyr Xaa Xaa Xaa Lys Xaa Xaa Xaa Xaa Xaa Xaa
130 135 140

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
145 150 155 160

Xaa Xaa Asn Xaa Xaa Gly Xaa His Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
165 170 175

Xaa Xaa Xaa Pro Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
180 185 190

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Gly Xaa Gly Xaa Xaa Xaa Arg Xaa
195 200 205

Xaa Xaa Xaa Xaa Xaa Asp Xaa Ala Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
210 215 220

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
225 230 235 240

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Xaa Xaa Leu Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/11576

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C12P 19/00, 17/04; C12N 1/12, 1/20, 5/00, 5/04
US CL :435/72, 126, 252.1, 252.3, 410, 419
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/72, 126, 252.1, 252.3, 410, 419

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, MEDLINE, EMBASE, BIOSIS, SCISEARCH, BIOTECHDS, NTIS, WPIDS, HCAPLUS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 85/01745 A1 (KRAFT, INC.) 25 April 1985 (23.04.85), see the entire document specially ages 4-7.	1-72
Y	NIKISHIMI et al. Occupance in Yeast of L-Galactonolactone Oxidase which is similar to a key enzyme for Ascorbic Acid biosynthesis in animals, L-Gulonolactone Oxidase. Arch. Biocem. Biophys. December 1978, Vol. 191, No. 2, pages 479-486, see the entire article, specially abstract and introduction sections.	1-72
A,P	WO 99/33995 A1 (ASCORBX LIMITED) 08 July 1999 (08.07.99), see the entire article.	1-72

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 AUGUST 1999	22 OCT 1999
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer MARYAM MONSHIPOURI JOYCE BRIDGERS PAR[illegible] SPECIALIST CHEMICAL MATRIX Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)★

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/11576

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/11576

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This international Preliminary Examining Authority has found 2 inventions claimed in the International application covered by the claims indicated below:

Group I, claims 1-59 and 71, drawn to a method of producing ascorbic acid or esters thereof in a microorganism comprising culturing a microorganism having a genetic modification to increase the action of an enzyme selected from the group consisting of hexokinase, glucose phosphate isomerase etc. as well as a microorganism genetically modified for producing ascorbic acid.

Group II, claims 60-70 and 72, drawn to a plant for producing ascorbic acid or esters thereof, wherein said plant has a genetic modification to increase the action of an enzyme selected from the group consisting of hexokinase, glucose phosphate isomerase etc.

The inventions listed as Groups I-II do not relate to a single inventive concept because they are considered to be two different categories of invention and are not drawn to combination of categories (i.e. categories 1-5), specified in 37 CFR section 1.475(b).